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## CHEMICAL CHANGES OF THE PAPAYA PLANT DURING DEVELOPMENT, WITH SPECIAL REF- ERENCE TO ITS PROTEOLYTIC ACTIVITY

by

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### INTRODUCTION

The papaya plant (*Carica papaya* L.) attains full maturity in approximately thirteen months of growth, when it bears fruit. During this period of development, several chemical changes take place in the plant. The present investigation v  
general trend of variation in  
of the plant throughout this  
were collected at the same t  
in moisture content, hydrogen ion concentration, and total nitrogen. As  
no data of this nature have previously been recorded, we have thought  
same may be of use to those engaged in the study of the papaya plant. For  
this reason, these data have been included in the present paper, together  
with those finding related to the distribution of proteolytic activity  
throughout the plant.

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### MATERIALS AND METHODS

In the present investigation, the following material and methods  
were used.



*Plant material.* The native variety of papaya was utilized. Seeds were sterilized and planted in a greenhouse. The seedlings were then transplanted into pots and, on attaining an age of approximately three months, were in turn placed out in the open. Samples for analysis were taken as recorded in Table I.

TABLE I

*Schedule Followed In Collecting Samples for Analysis*

MONTH AND YEAR	AGE IN DAYS AFTER PLANTING SEED	SAMPLE OF	AMOUNT OF SAMPLES COLLECTED
Sept. 1940	0	Seeds	50 gm.
" 1940	3	"	50 "
" 1940	6	"	50 "
" 1940	13	Whole seedling	50 seedlings
Oct. 1940	43	Whole plant	25 plants
Nov. 1940	73	" "	5 "
Dec. 1940	103	" "	2 "
Jan. 1941	133	" "	1 "
Feb. 1941	163	" "	1 "
Mar. 1941	193	" "	1 "
Apr. 1941	223	" "	1 "
May 1941	253	" "	1 "
June 1941	283	" "	2 "
July 1941	313	" "	1 "
Aug. 1941	343	" "	2 "
Sept. 1941	373	" "	1 "
Oct. 1941	403	" "	1 "

All of the samples were collected early in the morning and immediately sent to the laboratory, where they were placed in cold storage at a temperature of about 8°C. Samples were always utilized within the following twenty-four hours.

*Total moisture.* Total moisture was determined in the seed, root, stem, leaf, and fruit by placing samples of these various parts in an oven at a temperature of 110°C, until constant weight was attained. The loss in weight was taken as an index of the moisture content of each sample.

*Expression of juice.* The tissues were thoroughly ground with a small amount of washed quartz in a porcelain mortar and the paste, thus formed, squeezed in a piece of cheesecloth until all the juice was expressed. The juice obtained was used immediately.

*Hydrogen ion concentration.* The hydrogen ion concentration of the juice was determined with a Leeds and Northrup glass electrode meter.

*Milk clotting activity.* The milk clotting activity of the juice was one of the indexes used to measure proteolytic activity and the method followed in this determination, was that of Balls and Hoover (<sup>2</sup>). Results obtained in these milk tests were expressed in milk clotting units equivalent to the amount of enzyme required to clot 5 cc. of the standard milk preparation in one minute at 40°C.

*Hydrolysis of gelatin.* Another index used to measure proteolytic activity was the ability shown by the juice to hydrolyze gelatin; the method followed was the formol titration. A two per cent gelatin solution pH 5 was used as substrate. To 5 cc. of this solution 1 cc. of juice was added. One cc. of this mixture was taken for the initial titration. One cc. of 40 per cent formaldehyde was added to each volume of mixture to be titrated. The indicator used was a solution of 0.2 per cent phenolphthalein in 50 per cent alcohol. The incubation period lasted 20 hours at a temperature of 40°C. The strength of the sodium hydroxide solution used for titration was of 0.01N.

*Nitrogen content of the tissues examined.* All nitrogen determinations were performed in accordance with the Kjeldahl-Gunning-Arnold method (<sup>1</sup>).

#### EXPERIMENTAL RESULTS

*The seed.* No measurable amount of proteolytic activity was detected in the seeds before or during the germination period. The nitrogen content of the seed, as planted, was 41.5 mg. per gram of dry seed; samples taken during the thirteen days before germination averaged 44.4 mg. of nitrogen per gram of dry seed. For other determinations performed on the seed, see Table II.

*The leaf.* Proteolytic activity appeared first in the leaf and then in other parts of the plant as can be seen in Tables III, IV, and V. Samples collected forty-three days after planting the seed showed, on dry basis, a milk clotting activity of 1.80 units per gram and a formol titration of 15 cc. of 0.01N NaOH. The peak of proteolytic activity in the leaf, as indicated both by the milk clotting and formol titration, was reached about the 133rd day after planting of the seed. These values were 11.16 milk units and 56.16 cc. 0.01N NaOH, respectively, on dry basis per gram. From this date on, proteolytic activity of the leaf consistently decreased. The last test performed on the 403rd day gave 4.68 units and 13.61 cc.

0.01N NaOH per gram of dry leaf for the milk clotting test and formol titration, respectively. Nitrogen content of the leaf averaged 42.6 mg. per gram of dry leaf. The highest content was observed on the 253rd day, 49.9 mg., while the lowest, 35.6 mg., was recorded on the 193rd day. For other determinations performed on the leaf, see Tables II, III, and VI.

*The stem.* Milk clotting activity did not appear in the stem until the 73rd day. This initial activity, as well as that exhibited all throughout the experimental period, was much lower than in the leaf. Average clotting per gram of dry tissue was 2.48 milk units, while the formol titration was 8.18 cc. of 0.01N NaOH. The peak in milk clotting activity, 5.09 units, was reached on the 133rd day. On other hand, formol titration did not show a maximum until the 283rd day when a titration of 13.21 cc. of 0.01N NaOH per gram of dry stem was recorded. Average nitrogen content was 13.7 mg. per gram of dry tissue. A maximum of 20.8 mg. was recorded on the 253rd day and a minimum of 6.6 mg. on the 403rd day. For other data related to the stem, see Tables II, IV, and VI.

*The root.* Of the various parts of the papaya plant, the root showed the lowest milk clotting activity, although its gelatin hydrolyzing power was the same as that of the stem. Its average milk clotting activity and formol titration per gram of dry tissue were 1.39 milk units and 8.12 cc. of 0.01N NaOH, respectively. The peak in milk clotting activity, 3.43 units, was reached on the 283rd day; the peak in formol titration, on the same day with a value of 17.50 cc. of 0.01N NaOH. Average nitrogen content of the root was 14.3 mg. per gram of dry tissue; its maximum content was recorded on the 223rd day with 19.8 mg. and the lowest on the 373rd day, with 8.5 mg. per gram of dry tissue. For other data on this part of the plant, see Tables II, V, and VI.

*The fruit.* For the purpose of this study the fruit was divided into two parts: the rind and the pulp. It is well known that the rind contains the largest amount of the latex present in the fruit, while the pulp contains practically none. The fruit was collected at the same time from two different trees, both about thirteen months old. That fruit belonging to the younger inflorescence at the top was small and undeveloped; that from the older inflorescences at the bottom was well developed and medium ripe. Tables VII, VIII, IX, and X, record the inflorescences from the seventh to the first; the seventh being the younger at the top and the first being the older at the bottom of the tree.

(a) *The rind.* The average proteolytic activity of the rind per gram of dry tissue was 36.2 milk units and 26.4 cc. of 0.01N NaOH. Maximum

activity was observed in the fruit belonging to the third inflorescence with values of 94.1 milk units and 36.1 cc. of 0.01N NaOH, respectively, for milk clotting and formol titration. The fruit from this inflorescence can be seen from Table VIII. The average nitrogen content of the rind was of 29.9 mg. per gram of dry tissue. For other data, see Tables VII, VIII and X.

(b) *The pulp.* The pulp of the fruit showed a much lower proteolytic activity than the rind, both when such activity was measured by the milk clotting test as well as by formol titration. Milk clotting activity of the pulp was nearly one-tenth that of the rind (36.2 units against 3.3 units per gram of dry tissue). Formol titration of the pulp was about a one-third that of the rind (26.4 cc. against 9.9 cc. 0.01N NaOH per gram of dry tissue). The milk clotting power of the pulp from the second and first inflorescences was zero. On the other hand, its gelatin hydrolytic activity remained quite uniform throughout, showing an average of 9.9 cc. of 0.01N NaOH per gram of dry tissue. The average nitrogen content of the pulp was 14.9 mg. per gram of dry tissue, that is, about half the average nitrogen content of the rind. As in the rind, this nitrogen content also had a tendency to decrease with the age of the fruit. For other data on the pulp, see Tables VII, IX, and X.

#### DISCUSSION

Outside of the observations made by Balls, Thompson and Jones (<sup>3</sup>) to the effect that the entire papaya plant, aside from the roots, contained considerable enzyme activity, no other investigation, as far as we know, has been conducted along these lines.

The results obtained by us confirmed the observations of the above mentioned investigators and showed, furthermore, that milk clotting activity made its appearance first, in the leaf of the young plant and later, in the stem and root. The two parts of the plant which at all times showed a high proteolytic activity were the green leaves and the green fruit rind. Whether the formation of papain is closely connected with the presence of chlorophyll we are not in a position to say; however, it is interesting to note the close association that exists between these two substances in the different parts of the papaya plant.

In the leaf, at least, there was a definite variation in proteolytic activity during the first thirteen months of growth. Such activity reached its maximum value on the 133rd day after planting of the seed, but decreased consistently thereafter until the end of the experiment. The same variation was noticed, to a lesser extent, in the stem and in the root.

It must be remembered that these results are subject to considerable variation due to the use of single tree samples for each set of determinations. Lack of planting space made it necessary to take single trees for analysis in such a long time experiment. We feel, however, that in spite of individual variations, these results show definite trends in the distribution and activity of the substances measured.

#### SUMMARY

1. All parts of the papaya plant, with the exception of the seed, showed varying degrees of proteolytic activity.

2. Milk clotting activity appeared first in the leaf and later, in the stem and root.

3. The largest amount of proteolytic activity was concentrated in the green fruit rind and, in decreasing quantities, in the leaf, fruit pulp, stem, and root.

4. In the leaf and, to a lesser extent, in the stem and root, a definite variation took place in proteolytic activity during the thirteen initial months of growth. Maximum activity was reached between the fourth and ninth months.

5. Data have been collected regarding the moisture, hydrogen ion concentration, and nitrogen content of the various parts of the papaya plant during its initial thirteen months of growth.

#### SUMARIO

1—Todas las partes de la planta de la papaya, con excepción de la semilla, demostraron poseer distintos grados de actividad proteolítica.

2—La actividad proteolítica apareció primero en la hoja y más tarde en el tallo y las raíces.

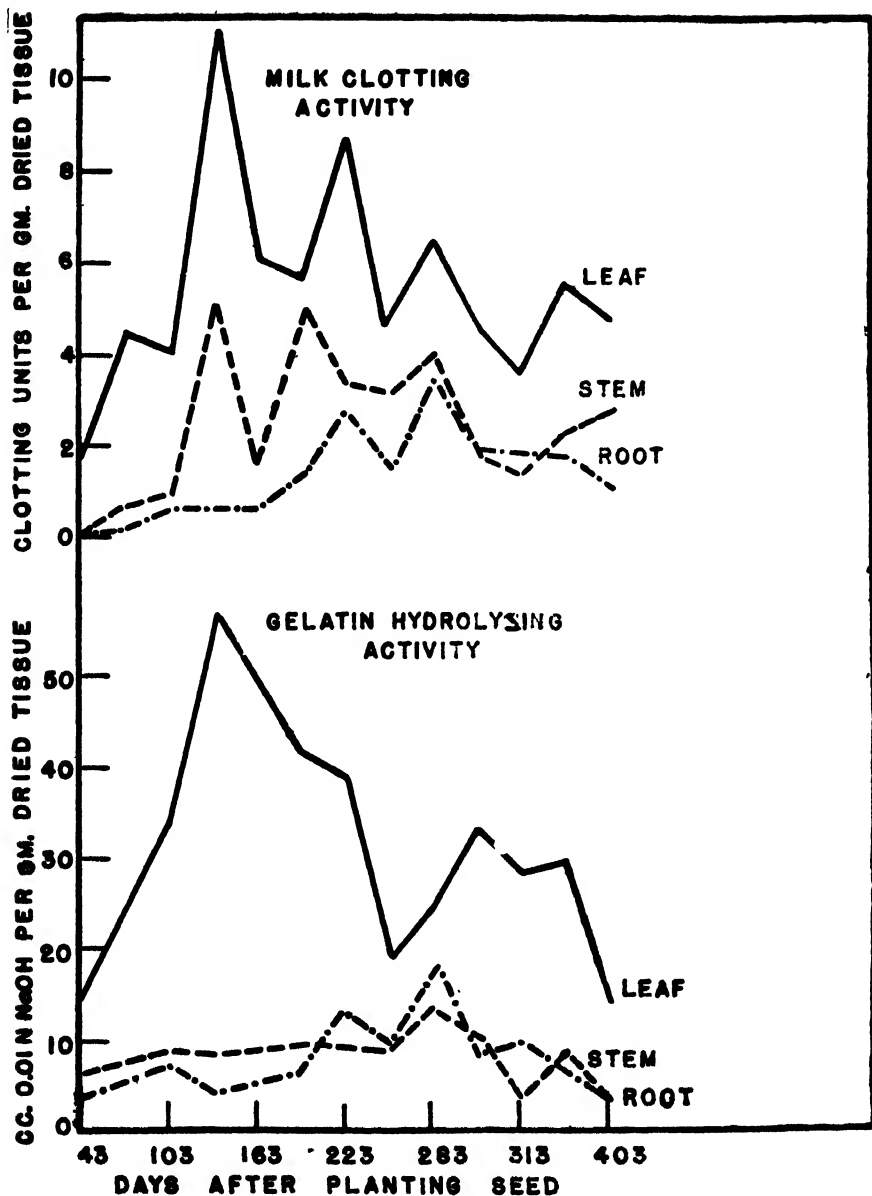
3—La actividad proteolítica estaba concentrada en mayor grado en la corteza de la fruta verde y, en cantidades menores, en las hojas, la pulpa de la fruta, el tallo y las raíces.

4—En las hojas y, en menor grado, en el tallo y las raíces, se produjo una variación definida en la actividad proteolítica durante los 13 meses iniciales de crecimiento. La actividad máxima se alcanzó entre los 4 a 9 meses.

5—Se han tomado datos en cuanto al contenido de humedad, variación en el pH y el contenido de nitrógeno de las distintas partes de la planta durante los 13 primeros meses de su desarrollo.

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2. Balls, A. K. Jour. Biol. Chem. 121: 737-745. 1937.
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*Milk clotting and gelatin hydrolyzing activity of the leaf, stem and root of the papaya plant during the first 403 days of growth.*

Table II. *Total moisture and total solids in the root, stem, and leaf of the papaya plant.*

AGE IN DAYS	TOTAL MOISTURE			TOTAL SOLIDS		
	ROOT %	STEM %	LEAF %	ROOT %	STEM %	LEAF %
43	86.5	92.8	87.8	13.5	7.2	12.2
73	86.5	88.3	82.5	13.5	11.7	17.5
103	88.5	90.0	84.4	11.5	10.0	15.6
133	89.0	95.5	92.3	11.0	4.5	7.7
163	89.9	86.5	66.0	10.1	13.5	34.0
193	89.7	93.5	75.0	10.3	6.5	25.0
223	90.0	88.0	75.7	10.0	12.0	24.3
253	90.5	87.2	78.8	9.5	12.6	21.2
283	91.2	87.6	79.1	8.8	12.4	20.9
313	87.0	84.9	70.2	13.0	15.1	29.8
343	88.5	80.7	71.2	11.5	19.3	28.1
373	89.9	87.5	78.8	10.1	12.5	21.2
403	87.0	86.5	78.0	13.0	13.5	22.0
AVERAGE	89.0	89.0	78.5	11.0	11.0	21.5

Table III. *Milk clotting and formol titration per cc. of leaf juice, per gram of dry leaf and per gram of fresh leaf.*

AGE IN DAYS	PH OF LEAF JUICE	MILK CLOTTING UNITS			FORMOL TITRATION CC. 0.01N NaOH			
		PER CC. OF JUICE	PER GM. OF DRY LEAF	PER GM. OF FRESH LEAF	PER CC. OF JUICE	PER GM. OF DRY LEAF	PER GM. OF FRESH LEAF	
43	6.10	0.25	1.80	0.22	2.08	15.00	1.83	
73	6.35	0.97	4.56	0.80				
103	5.77	0.76	4.10	0.64	6.18	33.37	5.22	
133	5.70	0.93	11.16	0.86	4.68	56.16	4.32	
163	6.58	3.17	6.15	0.21				
193	6.38	1.89	5.67	1.42	13.88	41.64	10.41	
223	6.09	2.79	8.64	2.11	12.43	38.53	9.41	
253	5.78	1.28	4.75	1.01	5.00	18.60	3.94	
283	6.06	1.69	6.42	1.34	6.39	24.28	5.05	
313	6.20	1.90	4.56	1.33	13.90	33.36	9.76	
343	5.95	1.44	3.60	1.03	11.20	28.00	7.90	
373	5.90	1.48	5.48	1.17	7.90	29.23	6.23	
403	5.98	1.30	4.68	1.01	3.78	13.61	2.95	
AVERAGE	6.06	1.53	5.50	1.01	7.95	30.15	6.09	



Table IV. *Milk clotting and formol titration per cc. of stem juice, per gram of dry stem and per gram of fresh stem.*

AGE IN DAYS	pH OF STEM JUICE	MILK CLOTTING UNITS			FORMOL TITRATION		
		PER CC. OF JUICE	PER GM. OF DRY STEM	PER GM. OF FRESH STEM	PER CC. OF JUICE	PER GM. OF DRY STEM	PER GM. OF FRESH STEM
43	5.75	0.00	0.00	0.00	0.47	6.06	0.44
73	5.80	0.09	0.68	0.08			
103	5.40	0.11	0.99	0.10	1.00	9.00	0.90
133	4.30	0.24	5.09	0.23	0.40	8.48	0.38
163	5.50	0.27	1.73	0.23			
193	5.88	0.35	5.04	0.33	0.68	9.79	0.67
223	5.46	0.46	3.36	0.41	1.25	9.13	1.10
253	5.31	0.42	3.17	0.37	1.30	8.97	1.14
283	5.58	0.49	3.96	0.43	1.86	13.21	1.63
313	5.60	0.34	1.90	0.29	1.88	10.53	1.60
343	5.62	0.32	1.35	0.26	0.77	3.23	0.62
373	5.55	0.33	2.31	0.29	1.20	8.40	1.05
403	5.55	0.43	2.75	0.37	0.50	3.20	0.43
AVERAGE	5.48	0.30	2.48	0.26	1.03	8.18	0.91

Table V. *Milk clotting and formol titration per cc. of root juice, per gram of dry root and per gram of fresh root.*

AGE IN DAYS	PH OF JUICE	MILK CLOTTING UNITS			FORMOL TITRATION CC. 0.01N NaOH		
		PER CC. OF JUICE	PER GM. OF DRY ROOT	PER GM. OF FRESH ROOT	PER CC. OF JUICE	PER GM. OF DRY ROOT	PER GM. OF FRESH ROOT
43	5.90	0.00	0.00	0.00	0.55	3.52	0.48
73	5.84	0.03	0.19	0.03			
103	5.93	0.09	0.69	0.08	0.68	5.24	0.60
133	5.00	0.09	0.73	0.08	0.55	4.45	0.49
163	5.47	0.07	0.62	0.06			
193	5.62	0.16	1.39	0.14	0.73	6.35	0.66
223	5.50	0.31	2.79	0.28	1.43	12.90	1.24
253	5.55	0.17	1.61	0.15	1.00	9.50	0.91
283	5.55	0.33	3.43	0.30	1.68	17.50	1.53
313	5.50	0.29	1.94	0.25	1.33	8.90	1.16
343	5.55	0.25	1.92	0.22	1.28	9.84	1.14
373	5.55	0.20	1.78	0.18			
403	5.28	0.16	1.07	0.14	0.50	3.34	0.44
AVERAGE	5.56	0.17	1.39	0.15	0.97	8.12	0.87

Table VI. *Milligrams of nitrogen per gram of fresh and dry leaf, stem, and root.*

AGE IN DAYS	MILLIGRAMS OF N PER GRAM OF FRESH TISSUE			MILLIGRAMS OF N PER GRAM OF DRY TISSUE		
	LEAF	STEM	ROOT	LEAF	STEM	ROOT
43	5.6	1.3	2.0	47.7	18.4	14.5
73	7.9	1.5	2.5	45.0	12.4	18.4
103	6.0	1.8		38.5	18.2	
133	2.9	0.7	2.1	38.0	15.2	18.7
163	15.2	2.5	1.5	44.8	18.5	14.4
193	8.9	0.8	1.3	35.6	13.0	13.3
223	10.8	1.6	2.0	44.3	13.4	19.8
253	10.6	2.6	1.5	49.9	20.8	15.8
283	9.2	1.6	1.5	44.2	13.0	16.7
313	12.7	1.4	1.3	42.5	9.0	10.0
343	13.0	1.8	1.1	46.5	9.4	9.4
373	8.4	1.2	0.9	39.8	9.9	8.5
403	8.7	0.9	1.6	39.3	6.6	12.6
AVERAGE	9.2	1.5	1.6	42.6	13.7	14.3

Table VII. *Total moisture and total solids in the rind and pulp of papayas of different inflorescences.*

INFLORESCENCE (FROM TOP TO BOTTOM)	RIND		PULP	
	TOTAL MOISTURE %	TOTAL SOLIDS %	TOTAL MOISTURE %	TOTAL SOLIDS %
7 younger	92.4	7.6	90.9	9.1
6	89.1	10.9	92.4	7.6
5	88.8	11.2	91.5	8.5
4	87.9	12.1	91.5	8.5
3	90.0	10.0	92.3	7.7
2	88.6	11.4	93.5	6.5
1 older	85.6	14.4	90.4	9.6
AVERAGE	88.9	11.1	91.8	8.2

Table VIII. *Milk clotting and formol titration per cc. of fruit rind juice, per gram of dry fruit rind and per gram of fresh fruit rind.*

INFLORESCENCE (FROM TOP TO BOTTOM)	pH OF JUICE	MILK CLOTTING UNITS				FORMOL TITRATION CC. 0.01N NaOH			
		PER CC. FRUIT RIND JUICE	PER GM. DRY RIND	PER GM. FRESH RIND		PER CC. FRUIT RIND JUICE	PER GM. DRY RIND	PER GM. FRESH RIND	
7 younger	5.38	1.83	22.3	1.69		2.94	24.2	2.62	
6	5.33	1.60	13.2	1.43		3.41	26.9	3.03	
5	5.28	2.28	18.0	2.03		3.39	24.8	2.98	
4	5.28	4.00	29.2	3.52		4.01	36.1	3.61	
3	5.15	10.45	94.1	9.41		3.52	27.5	3.12	
2	5.28	5.93	46.3	5.25		3.22	19.0	2.76	
1 older	5.08	5.13	30.3	4.39					
AVERAGE	5.25	4.46	36.2	3.96		3.42	26.4	3.02	

Table IX. *Milk clotting and formol titration per cc. of fruit pulp juice, per gram of dry fruit pulp and per gram of fresh fruit pulp.*

INFLORESCENCE (FROM TOP TO BOTTOM)	PH OF JUICE	MILK CLOTTING UNITS			FORMOL TITRATION CC. 0.01N NaOH		
		PER CC. FRUIT PULP JUICE	PER GM. OF DRY PULP	PER GM. FRESH PULP	PER CC. FRUIT PULP JUICE	PER GM. OF DRY PULP	PER GM. FRESH PULP
7 younger	5.53	0.30	3.0	0.27			
6	5.53	0.56	6.8	0.52	1.03	12.5	0.95
5	5.65	0.28	3.0	0.26	0.73	7.9	0.68
4	5.49	0.25	2.7	0.23	0.67	7.2	0.61
3	5.43	0.65	7.7	0.60	1.15	13.7	0.11
2	5.70	0.00	0.0	0.00	0.82	11.7	0.77
1 older	5.45	0.00	0.0	0.00	1.52	14.3	1.37
AVERAGE	5.53	0.29	3.3	0.27	0.85	9.9	0.75

Table X. *Milligrams of nitrogen per gram of fresh and dry fruit rind and pulp.*

INFLORES- CENCE (FROM TOP TO BOTTOM)	MILLIGRAMS OF N PER GRAM OF FRESH TISSUE		MILLIGRAMS OF N PER GRAM OF DRY TISSUE	
	RIND	PULP	RIND	PULP
7 younger	2.5	2.1	38.2	22.9
6	2.3	1.5	25.4	16.5
5	2.7	1.4	30.3	15.5
4	2.8	1.3	31.7	13.9
3	3.1	1.2	34.2	13.1
2	2.7	1.0	30.5	10.2
1 older	1.6	1.1	18.1	11.9
AVERAGE	2.7	1.4	29.9	14.9

Table XI. *Distribution of Proteolytic Activity and Nitrogen in the Papaya Plant.*

PART OF THE PLANT	PROTEOLITIC ACTIVITY PER GRAM OF DRY TISSUE		MILLIGRAMS OF NITROGEN PER GRAM OF DRY TISSUE
	MILK CLOTTING UNITS	CC. 0.01N NaOH	
Seed	0.00	—	41.5
Root (Average 13 months)	1.39	8.12	14.3
Stem (Average 13 months)	2.48	8.18	13.7
Fruit pulp (Average 1st to 7th inflorescence)	3.30	9.90	14.9
Leaf (Average 13 months)	5.50	30.15	42.6
Fruit rind (Average 1st to 7th inflorescence)	36.20	26.40	29.9



# THE EFFECT OF STAKING AND PRUNING ON TOMATO PLANTS

BY ARTURO RIOLLANO

Staking and pruning tomato plants has been a general practice among the majority of the farmers devoted to truck or market gardening in various sections of Puerto Rico. While numerous trials have been carried elsewhere to determine the relative value of these practices, experimental evidence is lacking for this Island. These trials were established to determine the effects of staking and pruning Marglobe tomato plants on yields, size, quality, and earliness of fruits under prevailing conditions at the Isabela Irrigation District. It is hoped that the information presented in this paper will be in general applicable to other sections of the Island or, at least, that it will induce some vegetable growers to make more careful observations as to the relative merits of pruning and training tomato plants in their respective areas.

## MATERIALS AND METHODS

Two trials were conducted on Coto Clay soil with the Marglobe variety at the Isabela Substation during the fiscal year 1936-37. This variety of tomatoes is grown almost exclusively in Puerto Rico, either for the local market or for shipping to the Continent during the winter season. The first trial was started on August 1936 with four treatments, namely, (a) unstaked, (b) staked, (c) unstaked and pruned, and (d) staked and pruned. Each treatment was replicated nine times in randomized blocks. The land used for this trial was prepared for surface irrigation by the furrow system commonly known as the Hawaii system. Seed was sown on August 20 in seedbeds made in the open and the seedlings transplanted to the field on September 19. Plants were set by hand  $2\frac{1}{2} \times 4$  feet apart. Square plots  $\frac{1}{100}$ th acre in area were used. Thirty-five plants were set in each plot. 8-10-15 fertilizer was applied to the furrow at the rate of one ton per acre five days before transplanting. Irrigation was applied at the



approximate rate of one acre-inch once a week whenever rainfall was less than one inch in the preceding week. A total of six applications of artificial irrigation were made while the amount of natural rainfall amounted to 13.7 inches during the crop.

In all the staking treatments each plant was tied to a wooden stake driven into the ground about 10 inches and extending from 5 to 6 feet above the ground. Tying of the plants to the stakes was done with manila hemp twine. Six times during the season this operation was practiced in order to keep plants properly tied to the stakes.

In the pruning treatments, plants were pruned to a double stem and small shoots were removed as often as necessary. Pruning was done at weekly intervals and this operation was practiced 6 times during the crop. Eight applications of Bordeaux mixture 2-3-50 plus 2 lbs. of lead arsenate were made at weekly or ten-day intervals for the control of diseases and insects. Seven pickings of fruits were made: the first on November 20 and last on December 28.

Fruit from each plot was counted and classified by weight as follows: large, weighing above .3 pound; medium, weighing from .2 to .3 pound; and small, weighing less than .2 pound. Fruit with blemishes, malformations, disease symptoms or too small for marketing purposes was classified as unmarketable. The fruit was harvested green or just beginning to turn, a stage of maturity considered safe for long distance shipment.

The second trial was established in another field after the results of the first trial were known. It was started on January 17, 1937, the first picking being made on April 6 and the last on May 19. A similar procedure was followed except that only two treatments were included, staked and unstaked, and that each treatment was replicated 8 times. This second test was carried out during the dry season and consequently 10 applications of irrigation water were made when rainfall dropped to 8.25 inches during the crop season.

The results of the first trial were calculated by Fisher's (3) method of statistical analysis while those of the second trial were analyzed by Student's method using Love's (5) modification. In the latter case odds greater than 30:1 are considered significant.

## RESULTS

The results are presented in detail in tables 1 and 2. They have been reported in hundredweights per acre to facilitate the making of compari-

TABLE 1. *Effect of staking and pruning tomato plants on yield, size, and quality of fruit. First Trial.*

TREATMENT†	LARGE (100 lbs.)	MEDIUM (100 lbs.)	SMALL (100 lbs.)	MARKETABLE FRUITS (100 lbs.)	CULLS (100 lbs.)	TOTAL YIELD (100 lbs.)
Unstaked	16.1	57.0	18.3	91.4	10.6	102.0
Staked	12.3	44.5	16.6	73.4	7.3	80.7
Unstaked and pruned	7.4	32.2	15.0	54.6	9.1	63.7
Staked and pruned	5.0	29.3	12.0	46.3	5.8	52.1
Difference for significance at 5 per cent point	2.5	14.4	2.9	7.4	2.5	8.2
Difference for significance at 1 per cent point	3.4	19.6	3.9	10.8	3.4	11.1

TABLE 2. *Effect of staking tomato plants on yield, size, and quality of fruit.  
Second Trial. Yields are given in hundredweights per acre.*

TREATMENT	LARGE (100 lbs.)	MEDIUM (100 lbs.)	SMALL (100 lbs.)	MARKETABLE FRUITS (100 lbs.)	CULLS (100 lbs.)	TOTAL YIELD (100 lbs.)
Unstaked	52.9	63.6	19.0	138.5	17.2	155.7
Staked	43.6	57.1	15.7	116.4	11.8	128.2
Loss by staking	9.3	9.5	3.3	22.1	5.4	27.5
Per centage loss	17.5	14.3	17.4	16.0	31.4	17.4
Student's odds <sup>1</sup>	216:1	70:1	59:1	1999:1	184:1	1428:1

<sup>1</sup> Odds greater than 30:1 are considered significant.

sons in terms used by local growers. Yields obtained in these trials seem to be low if compared to those obtained in the Continent. However, these yields compare favorably with those reported by Colón Torres and Morales (1) as obtained by commercial growers in the Jayuya section of this Island.

In both trials staking reduced consistently and significantly yields of all classes of fruit when compared with the unstaked treatments. The effect of staking on total yield is decidedly detrimental when considering that this practice caused an average loss of 21.3 hundredweights of fruit per acre in the first trial and 27.5 hundredweights in the second trial. Staking increases slightly the percentage of marketable fruit but reduces greatly total yields. The apparent effect of staking upon quality of fruit is offset by the marked reduction on total yields of marketable fruit. Thus it will be observed that staking reduced the yield of marketable fruit by 18.0 hundredweights in the first trial and by 22.1 hundredweights in the second trial.

The combination of pruning and staking seems to reduce further, in a significant way, the yields of all classes of fruit. The unstaked and pruned treatment was inferior to the staked or unstaked treatment, but it was superior to the staked and pruned treatment where the lowest yields were recorded. The descending order of merit of marketable yields for unstaked, staked, and unstaked and pruned, staked and pruned will, therefore, run as follows: 91.4, 73.4, 54.6 and 46.3 hundredweights per acre respectively. For total yield the same trend is observed for the above treatments, namely, 102.0, 80.7, 63.7, and 52.1 hundredweights per acre, respectively. Considering total yields of marketable fruit, the effects of pruning and staking upon quality are significantly undesirable.

The average weight of all fruit harvested from the different treatments in both trials has been presented in table 3.

TABLE 3. *The effect of staking and pruning on size of fruit.*  
*Average weight of fruit by trials and treatments.*

	UNSTAKED	STAKED	UNSTAKED & PRUNED	STAKED & PRUNED
	(lbs.)	(lbs.)	(lbs.)	(lbs.)
First trial	0.233	0.234	0.215	0.213
Second trial	0.278	0.255		

In spite of the apparent tendency to reduce size of fruit when pruning or staking was practiced, the differences in weight recorded were not sig-

nificant. Furthermore, these differences in size must be larger in order to be of any commercial value.

The effect of staking and pruning on earliness may be studied by observing the total yields obtained with the different treatments in the first four pickings. In the first trial, as illustrated in figure 1 and table 4, it seems that pruning without staking had some favorable effect on the quantity of early fruit set by causing slightly higher yields in the

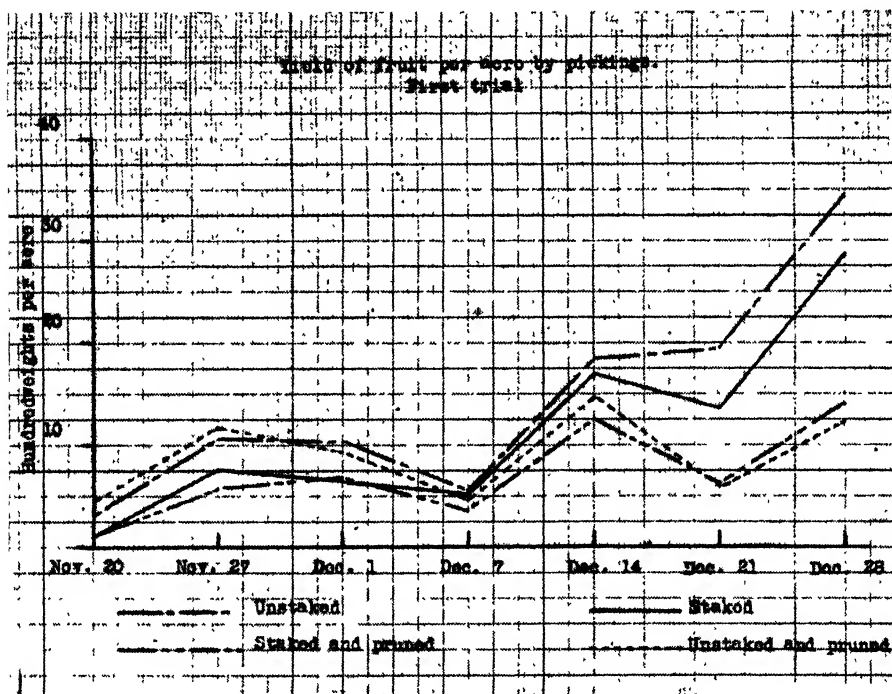


Fig. 1. The effects of pruning and staking on total yields and earliness of fruit.

pickings of November 20 and 27. When considering total yields of the first four pickings, the unstaked plots whether pruned or unpruned, were not statistically different, but each outyielded in a significant way the other two treatments. In the second trial where pruning treatments were omitted, the unstaked treatment with 106.6 hundredweights per acre in the first four pickings, had a marked and significant effect upon the quantity of early fruit set when compared with the staked treatment which produced only 75.1 hundredweights of fruit per acre during the same

TABLE 4. *Effect of staking and pruning on earliness. First Trial.*  
*Yields in hundredweights per acre by pickings.*

DATE	UNSTAKED	STAKED	UNSTAKED & PRUNED	STAKED & PRUNED	DIFFERENCE FOR SIGNIFI CANCE
	(100 lbs.)	(100 lbs.)	(100 lbs.)	(100 lbs.)	
November 20	3.3	1.3	4.2	1.4	
November 27	10.5	7.6	11.5	6.3	
December 1	10.3	6.8	9.7	7.1	
December 7	5.7	5.3	4.9	3.9	
Yields per acre first four pickings, 9 replicates	29.8	21.0	30.3	18.7	3.5 * 4.8 **
December 14	18.4	17.3	14.8	12.7	
December 21	19.3	13.7	6.3	6.5	
December 28	34.5	28.7	12.3	14.2	
Totals	102.0	80.7	63.7	52.1	8.2 * 11.1 **

\* Significance at 5 per cent point.

\*\* Significance at 1 per cent point.

TABLE 5. *Effect of staking on earliness. Second Trial.*  
*Yields in hundredweights per acre by pickings.*

DATE	UNSTAKED	STAKED	STUDENT'S ODDS
	(100 lbs.)	(100 lbs.)	
April 6	9.8	6.5	
April 14	31.6	19.0	
April 21	36.6	27.5	
April 28	28.6	22.1	
Yields per acre first four pickings. 8 replicates	106.6	75.1	4999:1
May 5	22.8	25.3	
May 12	13.4	14.4	
May 19	12.9	13.4	
Totals	155.7	128.2	1428:1

period of time. The results of both trials as shown in tables 4 and 5, indicate that staking is responsible for a significant reduction in the quantity of early fruit set.

Considering only the staked and unstaked treatments in both seasons, it will be observed in figures 1 and 2 that higher yields, regardless of treatment, were obtained during the last three pickings in the first trial which was established on August 1936; while in the second trial which

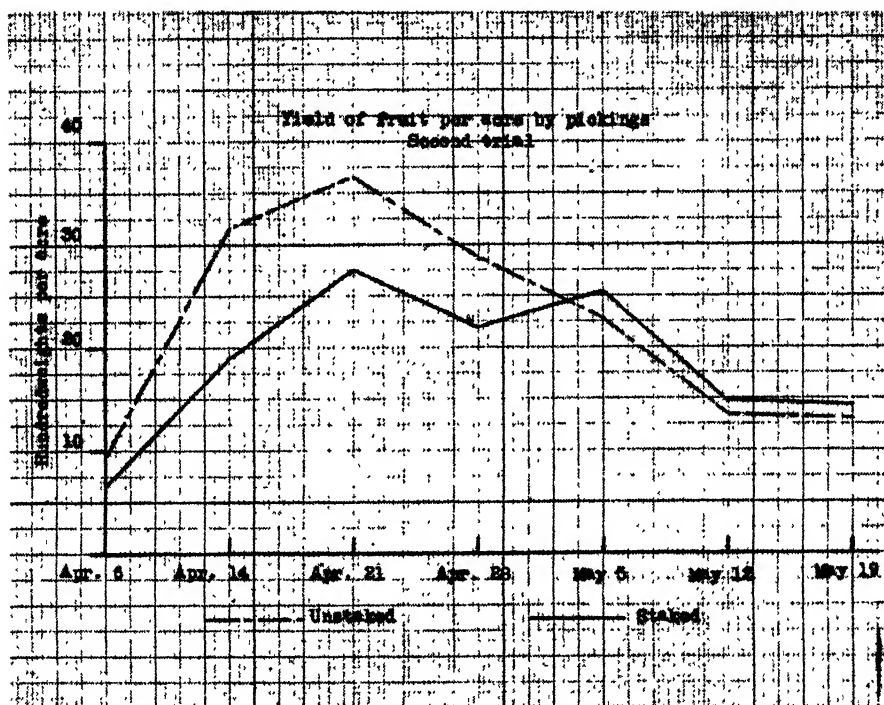


Fig. 2. The effects of staking on total yields and earliness of fruit.

was started on January 1937, the lowest yields were recorded in the last three pickings with the exception of the fifth picking of May 5th in the staked plots. This contrasted trends of yields might be attributed to seasonal effect.

#### DISCUSSION AND SUMMARY

The fact that these trials were established under irrigation by the furrow system must be clearly emphasized. In this way it seems that

the possibilities of increasing the amount of unmarketable fruit in the unstaked plants are greatly favored because a large percentage of the fruit clusters lie in direct contact with the water soaked soil when irrigation is practiced. The main argument offered by vegetable growers who stake and prune their tomatoes is that such cultural methods tend to increase marketable yields because the fruit clusters develop high above the soil, free from dirt. Nevertheless, the two trials conducted at the Isabela Substation show conclusively that pruning and staking tomato plants reduce considerably yields of marketable and total amount of fruit as long as the same number of plants are kept constant in the different practices compared. These results are in accord with the abundant evidence presented by Thompson (7) on these practices with trials he conducted in New York State and with the profuse literature he has reviewed on this subject. In the trials conducted in Australia Strickland (6) concluded that staking and pruning of unstaked plants were of doubtful value, except on limited areas.

However, the apparent conflicting results reported by other workers are due mainly to the use of less space or more plants per acre for the pruning and staking practices as compared to the untreated plots or, probably, to the wide variations in environmental conditions under which the different trials were established. Conflicting results may also follow according to Currence (2) when different varieties of tomatoes are subjected to pruning and training. He found that pruning was beneficial to Break O'Day variety and apparently detrimental to Pritchard. Even assuming that pruning and staking with twice the number of plants per acre as compared to the untreated plants, would produce equal or larger amounts of marketable fruit, the profitableness of such practices is still questionable. The added expenses involved in setting more plants per acre plus the cost of stakes, training and pruning several times during the crop, must be also considered before drawing general conclusions on the advisability of such cultural methods.

Pruning and staking had no favorable effects on the size and earliness of fruit in these two trials. In the second trial staking caused a significant decrease on the quantity of early fruit set when compared with the untreated plots. Earliness is not considered an important factor in Puerto Rico where tomatoes are grown successfully at any time of the year.

Hawthorne (4) has reported in Texas an increase in the amounts of early marketable yields from the first four pickings of pruned unstaked plants. This contrast with our results might be explained by the effect



of differences in climatic conditions. However, he found that when yields of the entire crop were considered, the unpruned plants resulted with the highest yields. Hawthorne also did not find any effect of pruning on size of fruit.

In conclusion, it may be stated that the results of our trials with pruning and staking Marglobe tomato plants plus the cumulative evidence from similar experiments conducted elsewhere, seem to indicate that such cultural methods are not conducive to the highest yields of marketable fruit. Pruning and staking under our climatic conditions have a tendency to decrease total marketable yields while not affecting favorably size, quality nor earliness of fruit.

### SUMARIO EN ESPAÑOL

#### ESTAQUEO Y PODA DE LOS TOMATES

El estaqueo y la poda en los tomates han sido prácticas muy generalizadas entre los agricultores que se dedican a la producción de hortalizas en Puerto Rico. Se cree que con estas prácticas se aumenta la producción y se consigue una proporción más alta de fruta de mejor calidad para la exportación o para el mercado local.

Con el fin de obtener información sobre el particular, se establecieron dos experimentos con tomates bajo regadío en la Subestación Experimental de Isabela en donde se compararon los siguientes tratamientos: (a) testigo sin estaqueo o poda, (b) estaqueo sin poda, (c) poda sin estaqueo y (d) estaqueo con poda. Se utilizó el mismo número de plantas de la variedad "Marglobe" en cada tratamiento y se tomaron las precauciones necesarias para que todos los tratamientos con ocho y nueve repeticiones recibieran atención uniforme.

Los resultados de la primera prueba indicaron que el estaqueo redujo la producción total de fruta comercial en un 20 por ciento aproximadamente y cuando se practicó la poda sin estaqueo, esta reducción llegó a un 40 por ciento comparado con el testigo sin poda ni estaqueo. Además, cuando se estaqueó y se podó, la reducción fué aún mucho mayor ya que la producción total bajó al 50 por ciento aproximadamente comparado con el testigo. En la segunda prueba en que se sometió la poda, se encontró que el estaqueo redujo la producción de fruta comercial en un 22 por ciento. Aunque el estaqueo en ambas pruebas causó un pequeño aumento en la proporción de fruta comercial, esta ventaja aparente quedó compensada por los efectos en la reducción total de fruta producida. En otras palabras, cuando no se estaqueó ni se podó, se produjo una mayor proporción de rezagos o fruta inservible para la venta; pero como la producción total de fruta aumentó grandemente, el total de fruta comercial producida fué entonces mucho mayor que cuando se estaqueó o se podó.

Ni el estaqueo ni la poda tuvieron efectos favorables en cuanto al tamaño de la fruta o la producción de una cosecha temprana se refiere. En términos generales estos resultados coinciden con los obtenidos en numerosas pruebas que se han llevado a cabo en diversas regiones de los Estados Unidos y en Australia. En conclusión puede decirse que nuestras pruebas de estaqueo y la poda en los tomates de la variedad "Marglobe", unida a la evidencia acumulada en pruebas efectuadas en otros sitios, demuestran que estas prácticas son perjudiciales ya que disminuyen la producción comercial del fruto y aumentan considerablemente los gastos de la cosecha.

# STUDIES OF THE SHADE REQUIREMENTS OF VANILLA

by

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## INTRODUCTION

The vanilla of commerce [*Vanilla fragrans* (Salish.) Ames] requires considerable shade for its best development. In existing vanilleries in Puerto Rico this shade is provided either by the use of the dwarf bucare (*Erythrina berteroana* Urban), which also serves as a support tree or by existing shade trees such as the guaba (*Inga vera* Willd.), and the guamá [*Inga laurina* (Sw.) Willd.]

Newport (5) states that the shade necessary for vanilla is not dense but rather chequered. According to Galbraith (2) in selecting trees for shade those should be chosen which give moderate to about half shade and which do not shed their leaves all at once. Chalot (1) points out the fact that weight and aroma of the beans are greatly influenced by the degree of sunlight to which the vines are exposed. Meinecke (4) states that in general the vanilla vine demands a little more sunlight than shade especially during the flowering season and at the time the beans are maturing. According to McClelland (3) the character of growth of vanilla vines depends greatly on the amount of shade to which the vines are exposed.

From the literature cited it is evident that some shade is essential for vanilla. Since no record could be found as to the exact amount required, an experiment was conducted at the Puerto Rico Experiment Station to determine this by observing the behavior of the vines when planted under different exposures to sunlight.

## EXPERIMENTAL PROCEDURE

The field used for this experiment had a gentle slope and the soil was a Catalina clay. Drainage ditches in lengthwise and crosswise directions divided the field into 16 contiguous plats each measuring 30 by 20 feet.

The four light exposures tested were as follows: full sunlight, two-thirds sunlight, one-half sunlight, and one-third sunlight. Exposures to light less than full were provided by overhead and side bamboo laths appropriately spaced. Each light treatment was replicated 4 times, and there were 30 plants in each replicate making a total of 120 plants per treatment or 480 plants in the entire experiment. Eight-node vanilla cuttings selected for uniform size and vigor were planted at the base of non-living support stakes. The usual mulching practice for vanilla was followed throughout.

All cuttings were planted on October 9, and every 3 months thereafter data were taken on root formation, seed-piece rotting, and vegetative growth. In addition, the condition of the vines at the termination of the experiment as well as their weights and the number of eight-node cuttings obtained were used to evaluate the various light treatments.

## EXPERIMENTAL RESULTS

*Root Formation*

With respect to the number of roots formed, it was found that the vines planted under one-third sunlight consistently produced the greatest

Table 1. *Cumulative root formation on vanilla cuttings planted under four exposures to light, Mayaguez, October 9, 1940.*

TREATMENT (EXPOSURE TO LIGHT)	ROOTS FORMED <sup>1</sup>			
	JAN 9	APRIL 9	JULY 9	OCT. 9
	NUMBER	NUMBER	NUMBER	NUMBER
Full sunlight -----	300	2 93	2 58	2 53
$\frac{2}{3}$ sunlight -----	352	2 319	2 303	2 300
$\frac{1}{2}$ sunlight -----	394	410	449	472
$\frac{1}{3}$ sunlight -----	428	451	498	2 490

<sup>1</sup> On 120 8-node cuttings planted in each treatment.

<sup>2</sup> Decrease due to decay of some roots previously formed.

number of roots, while the vines planted under full sunlight produced the least. The data for root formation are presented in table I.

It is interesting to note that the number of roots formed on the cuttings of each treatment was inversely proportional to the degree of sunlight to which the cuttings were exposed.

### *Seed-piece Rotting and Stem Growth of Vines*

The data pertaining to seed-piece rotting and stem growth of vines were very interesting. The cuttings grown under one-half and one-third normal sunlight suffered the least amount of stem rotting. In addition, they produced consistently the greatest amount of stem growth. Although the difference between these two treatments was in favor of the vines grown under one-third normal sunlight it was by no means considerable. These results are shown in table II.

As shown in table 2 the vines exposed to full sunlight and two-thirds sunlight sustained the greatest amount of seed-piece rotting and produced the least amount of stem growth. It is clearly seen that the extent of rotting of the seed-pieces was in direct proportion to the degree of sunlight they received, namely, the less the sunlight the lower the percentage of rotting of the seed-pieces. In the case of the stem growth made by the cuttings the reverse was true, that is, the amount of vegetative growth was in inverse proportion to the exposure to light.

Figure 1 shows the cumulative amount of stem growth made by the vines under the different exposures to light at each recorded reading, together with the amount of rainfall measured between readings.

It is evident from figure 1 that at each recorded reading there was no great difference in amount of vegetative growth between the cuttings grown under one-half sunlight and those grown under one-third sunlight. However, there were considerable differences in this respect which were statistically significant between the two treatments of more and less than one-half sunlight. It is of interest to note in figure 1 also that the greatest amount of vegetative growth between readings occurred during periods of abundant rainfall.

### *Condition of Vines and Weight and Number of Cuttings*

At the termination of the experiment the condition of the vines under the various exposures to light was recorded to determine how the various

Table 2. *Extent of rooting and stem growth made by vanilla cuttings planted under four exposures to light, Mayagüez, October 9, 1940.* <sup>1</sup>

TREATMENT (EXPOSURE TO LIGHT)	JANUARY 9		APRIL 9		JULY 9		OCTÓBER 9		JANUARY 9	
	INTERNODES DECAYED	STEM GROWTH	INTERNODES DECAYED	STEM GROWTH	INTERNODES DECAYED	STEM GROWTH	INTERNODES DECAYED	STEM GROWTH	INTERNODES DECAYED	STEM GROWTH
	PERCENT	FEET	PERCENT	FEET	PERCENT	FEET	PERCENT	FEET	PERCENT	FEET
Full sunlight -----	18.6	0.62	66.7	1.54	83.3	2.41	89.5	2.34	92.1	5.89
¾ sunlight -----	1.5	0.80	13.5	2.36	35.7	5.07	38.1	9.73	44.3	16.17
½ sunlight -----	1.4	1.13	2.3	3.45	3.2	7.81	4.8	15.76	7.7	22.67
¼ sunlight -----	0.01	1.13	1.3	3.52	2.0	8.27	4.2	15.90	7.1	23.94

<sup>1</sup> On 120 8-node cuttings planted in each treatment. Data are cumulative.

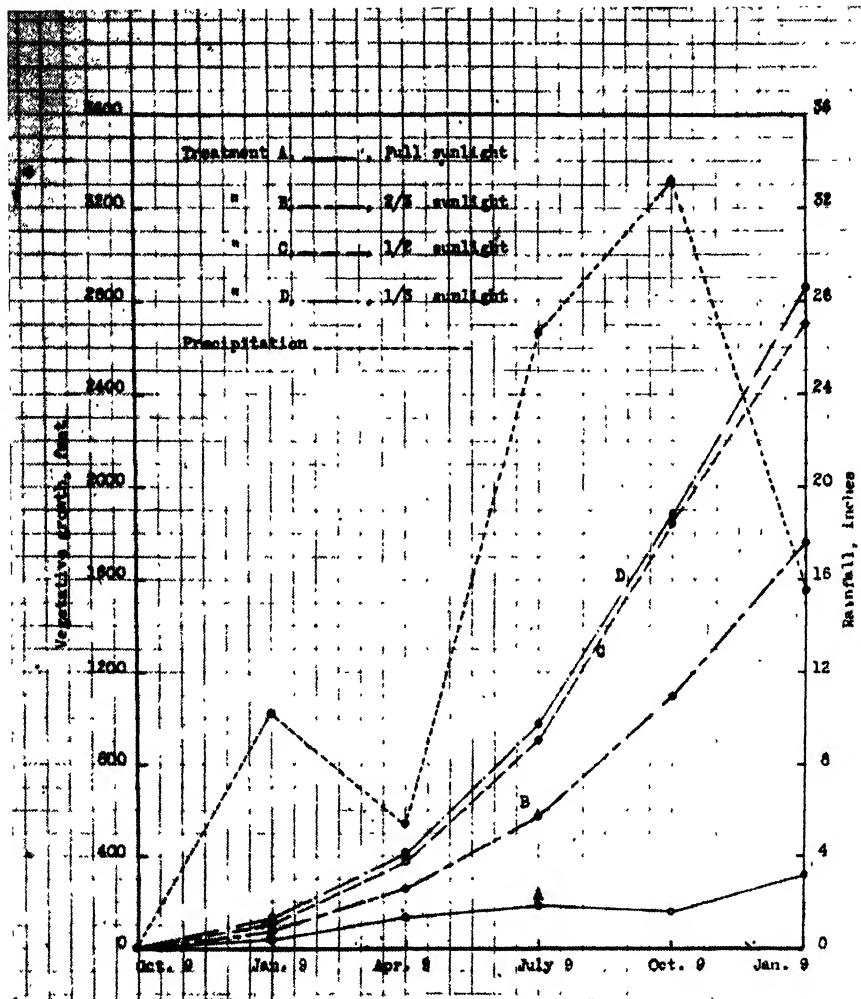


Figure 1. Vegetative growth made by vanilla cuttings at 3-month periods when grown under four exposures to sunlight, together with the amount of rainfall between readings.

degrees of sunlight had affected the development of the vines. This information is summarized in table 3 together with the weight of the vines and number of eight-node cuttings obtained from those grown in each treatment.

The vines grown under one-half and one-third normal sunlight produced the highest percentages of healthy plants and the least percentages

Table 3. *Condition of vanilla plants at end of 15 months planted under four exposures to light, together with weight of vines and number of 8-node cuttings per treatment.*

TREATMENT (EXPOSURE TO LIGHT)	CONDITION OF PLANTS			WEIGHT OF VINES	EIGHT-NODE CUTTINGS
	HEALTHY	WILTED	DEAD		
	PERCENT	PERCENT	PERCENT	POUNDS	NUMBER
Full sunlight -----	32.5	16.7	50.8	26.8	142
$\frac{2}{3}$ sunlight -----	84.2	5.0	10.8	201.0	713
$\frac{1}{2}$ sunlight -----	98.3	0.0	1.7	344.0	975
$\frac{1}{3}$ sunlight -----	96.6	1.7	1.7	366.0	963

of wilted and dead plants. The same vines also produced the highest weights and greatest numbers of eight-node cuttings.

The vines grown under full sunlight produced the lowest proportion of healthy plants and highest proportion of wilted and dead plants. It is evident that there were considerable differences in the weights and numbers of eight-node cuttings between the vines exposed to the two lowest exposures to sunlight and those exposed to the two highest degrees of sunlight.

#### CHARACTER OF GROWTH OF VINES

Since the beginning of the experiment it was observed that the character of growth of the vines varied with the exposure to light to which they were exposed. The vines exposed to one-third and one-half the normal sunlight developed more vigorously, as illustrated in figure 2 and 3, than those exposed to two-thirds and full sunlight, as illustrated in figures 4 and 5. Moreover, the former were of a dark green color and their internodes longer and thicker, as compared to the yellow to yellowish-green and shorter and thinner internodes of the vines exposed to full and two-thirds sunlight.

#### SUMMARY

The work here reported showed that the vanilla plant requires considerable shade for its best development. When vanilla was grown under four exposures to light it was found that the best vine growth from the



Figure 2. Typical plants of *Vanilla fragrans* grown under one-third sunlight at the end of 15 months. Note the vigorous condition of the vines and the thick and long internodes. The vines were of a rich dark-green color.





Figure 3. Typical plants of *Vanilla fragrans* under one-half sunlight at the end of 15 months. The vines were vigorous and had long thick internodes, but the color was somewhat lighter than that of vines exposed to one-third normal sunlight.



Figure 4. *Typical plants of Vanilla fragrans grown under two-thirds sunlight at the end of 15 months. These vines were considerably less vigorous than those shown in figures 2 and 3. Also the internodes were shorter and thinner. The color was yellowish-green.*



Figure 5. *Typical plants of Vanilla fragrans grown under full sunlight at the end of 15 months. Note the drooping leaves and short internodes. Vines were yellow-green, almost chlorotic in color.*

standpoint of root germination, seed-piece rotting, and vegetative growth was accomplished under shade conditions which admitted from one-third to one-half the normal sunlight when the sun was directly overhead. The most vigorous and greatest weight of vines and the greatest number of eight-node cuttings were also obtained under those conditions.

### SUMARIO

Se indica en estudios sobre desarrollo vegetativo que la vainilla requiere abundante sombra. Sembrándose bajo cuatro distintas proporciones de sombra se notó el mejor desarrollo de las plantas cuando se sombreó en tal forma que se admitiera una tercera parte o una mitad de la cantidad de luz solar. Este desarrollo se expresó en producción de raíces, tallo y baja podredumbre de los esquejes. El mejor índice de desarrollo fué el número de esquejes de ocho nudos y la lozanía de las plantas.

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## NATURAL PARASITISM BY *TRICHOGRAMMA MINUTUM* OF THE EGGS OF THE SUGAR-CANE MOTH BORER, *DIATRAEA* *SACCHARALIS*, IN THE CANE FIELDS OF PUERTO RICO

By

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### THE STATUS OF *Diatraea*

The insect pest causing greatest injury to sugar-cane in Puerto Rico at the present time is the lesser moth borer, *Diatraea saccharalis* F. This was not true even a few years ago, when white grubs chewing into the root-stalks were much more seriously injurious, but since the giant Surinam toad, *Bufo marinus* L., was introduced and became abundant feeding on the adults of the white grubs, these insects have generally become so scarce as to be of minor importance at present. The weevil root-stalk borer, *Diaprepes abbreviatus* L., is also to some extent reduced in numbers by the introduced toad, and the injury caused by this insect alone (unaccompanied by that of white grubs) is rarely sufficiently noticeable to be observed by the cane grower. For a series of years following the introduction and planting of varieties of sugar-cane immune or resistant to mosaic disease, the yellow aphid, *Sipha flava* Forbes, became a serious pest on these varieties of cane, and often spread from them to adjacent fields of other varieties. In the last few years, however, further changes in the varieties of sugar-cane extensively grown have reacted so unfavorably on the yellow aphid that it now seems to be usually controlled naturally by rainfall and its predaceous and parasitic enemies. This leaves the moth



borer as the only remaining serious pest of sugar-cane in Puerto Rico not subject to more or less effective commercial control by natural factors.

The lesser moth borer occurs as a pest of sugar-cane not only in Puerto Rico, but also in all of the other West Indies (including all of the Lesser Antilles in which sugar-cane is grown), in Louisiana, Mexico, Brazil, Perú and Argentina, and is known to have been present in all these countries almost since the beginning of sugar-cane cultivation. Naturally it has been the subject of extensive studies, but without the discovery of any really effective control anywhere. A very partial control is obtained in Puerto Rico by the extensive adoption of the practice of not burning cane trash, before, during or after the harvesting of the cane stalks: excellent so far as it goes, but after all, not as effective as might be wished, and often erratic in its results. The lack of any effective artificial method of control has necessarily resulted in a very careful consideration of the use and value of the natural enemies of the moth borer.

#### PARASITES OF THE LARVA

Considering the natural enemies already present in Puerto Rico in the inverse order of their importance, it should be noted that the fungus attacking the caterpillars, *Cordyceps* (*Isaria*) *barberi* Giard, altho reported as being rather common in some of the other West Indies, is normally so scarce in Puerto Rico as to be valueless in control.

In examining the stomach contents of Puerto Rican lizards to determine what they had eaten, Wolcott (1924) noted that all of the common smaller species present in or near cane fields, *Anolis pulchellus*, *Anolis krugii*, *Anolis stratulus* and *Anolis cristatellus*, had eaten moth borer caterpillars, but in too small an amount to form a very considerable factor in control.

Concerning the Tachinid fly which attacks *Diatraea* caterpillars, Box (1928 a) states that "*Lixophaga diatraea* Townsend is the only larval parasite of any importance in Porto Rico." He found that the average parasitism during February-March 1925 was 12%, and during October-November 1926 was 37% (between Río Piedras and Trujillo Alto), but his conclusion as to how the value of this parasite might be increased for Puerto Rico is distinctly discouraging. "The writer does not think that the efficiency of *Lixophaga* can be artificially increased in any way, as it seems that this parasite has reached its maximum effectiveness." More recently, Dr. K. A. Bartlett (Anon. 1939), incidental to his work on rearing imported parasites of *Diatraea*, has collected extensive data on *Lixophaga*.

"The highest parasitism recorded for this species was 31.9 percent at Hormigueros, while the average for all collections was 9.6 percent. In view of the high infestation of cane by the moth borer, which was found to be as high as 96 percent in some fields on the south coast, it can be readily appreciated that on the whole this native parasite plays only a small part in the biological control of the borer. In some localities, however, under certain favorable conditions, it is of economic importance"

#### INTRODUCED PARASITES

The introduction from other countries into Puerto Rico of natural parasites of the moth borer not already occurring here was initiated by Mr. Harold E. Box (1924), when he brought from British Guiana in cold storage numerous cocoons of two Braconid wasps which attack the caterpillars: *Microdus diatraeae* Turner (now called *Bassus stigmaterus* Cresson) and *Ipobracon grenadensis* Ashmead. The former species became established here, for he recovered it in small numbers in 1925 and 1926 at Aguirre, where the releases were made, and it has since been found at Hormigueros by Dr. K. A. Bartlett in 1936, and by the writers at Isabela in 1938. It is by no means abundant in the other countries where it occurs, and presumably it is hardly to be expected that it will become sufficiently abundant in Puerto Rico to be an important factor in control. Despite the larger number of *Ipobracon grenadensis* later sent by Mr. Box (1928) and Mr. Luis A. Catoni from Venezuela, this species apparently did not become established here (Sefn 1929), or at least it has not since been recovered in the field.

In 1935, additional shipments of *Bassus stigmaterus* Cresson were made from British Guiana by Mr. S. M. Dohanian (1937), altho his efforts there were largely devoted to obtaining large numbers of the so-called "Amazon Fly", *Metagonistylum minense* Townsend, for release in Puerto Rico. In 1936, Mr. Dohanian sent large numbers of *Ipobracon rimac* Wolcott from Peru to Puerto Rico, but none has since been recovered here in the field. The Amazon Tachinid fly apparently did not become established at this time, and later Dr. Bartlett made additional shipments of the Amazon fly from British Guiana and reared large numbers in captivity in Puerto Rico for release here, but with no field recoveries. In 1939, Dr. Bartlett collected in Sao Paulo, Brazil, an abundance of material of a physiological strain of the Amazon fly occurring under comparatively dry conditions, which, it was thought, might be better adapted to the dryer regions of Puerto Rico. This strain has been reared in Puerto

Rico and large releases made, with field recoveries (Aaon 1940) at some localities.

Of the natural enemies, native and introduced, of the *Diatraea* caterpillar, one may summarize the prospect in Puerto Rico for the control of the moth borer, that (1) the fungus is of negligible value, (2) the lizards which eat the caterpillars are a minor factor, (3) the native Tachinid fly, which is unquestionably a considerable factor at times, can not be increased in abundance or efficiency by anything that can be done by man, (4) the one introduced parasite which has become established here is scarce, and, being of little importance elsewhere, may be presumed to be of little more value present or prospective in Puerto Rico, and (5) the status of the Amazon fly is still uncertain.

#### PARASITES OF PUPA AND ADULT

Specific parasites of the pupa of *Diatraea saccharalis* are unknown. One can only surmise as to natural enemies of the adult, for flight of the moths is almost entirely nocturnal, and, for practical purposes, is confined to the cane field. Lizards may possibly eat resting adults in the daytime, or at night those coming to rest around lights. No nocturnal birds occur in Puerto Rican cane fields, but possibly swallows at twilight might catch some of the moths. There is a distinct possibility that bats might catch and eat considerable numbers, but cane fields adjacent to bat caves give no indication of this by the smaller number of eggs laid on the leaves, or less stalk injury caused by the caterpillars. Indeed, the prospect of control of the moth borer by its natural enemies during the pupal and adult stages is by no means bright, leaving the egg stage as the one remaining point of attack.

#### THE EGGS OF *Diatraea*

The individual eggs of *Diatraea saccharalis* are oval and quite flat, being lenticular in cross-section, and are normally deposited in clusters consisting of rows usually paralleling the veins of the cane leaf, regularly overlapping like fish-scales, on either the upper or the lower side of the cane leaf. The junior writer found that less than 2% of all clusters collected over a period of some months are deposited close to the margin of the leaf; 31% were on the midrib of the upper surface of the leaf; 23% were beside the midrib on the under side of the leaf, and 45% were between the midrib and the outer margin of the leaf. The number of eggs in a cluster varies considerably, but four-fifths of the clusters contain be-

tween 8 and 36, altho large clusters containing between fifty and seventy are sometimes found, as are also individual eggs, or clusters of only two or three.

When first laid, the eggs are very light yellow, deepening somewhat in color for the next few days, becoming spotted with orange on the next to the last day, and showing the black head and the segmentation of the caterpillar thru the thin egg-shell by the final day before hatching. During extremely hot weather, incubation of eggs in the field may require as little as five days, but under ordinary conditions in the tropics, hatching takes place on the sixth or seventh day. Thus, for an entire week one stage of the borer is in plain sight on the cane leaves, where it can be readily attacked by any predaceous or parasitic animal interested in such a minute bit of concentrated nourishment.

#### TRICHOGRAMMA

The eggs of many different kinds of moths and butterflies in many tropical and temperate zone countries are attacked by a minute cosmopolitan wasp, *Trichogramma minutum* Riley. This Chalcid wasp has pink eyes, a yellow body, darker abdomen and clear, iridescent wings, and despite its minute size, is generally so abundant that entomologists, at least, often see it in the field parasitizing some moth or butterfly egg. It is so minute as to find ample nourishment for the complete development of one individual (and sometimes of two or more) to adult within the shell of a single moth or butterfly egg. It attacks the eggs of *Diatraea saccharalis*, and is so omnipresent as to constitute one of the most important factors in borer control. It is to favor this parasite that the non-burning of cane trash is advocated. Furthermore, as a more positive measure in increasing the abundance of this parasite in cane fields at the time when its presence is most essential, the release of large numbers of individuals artificially reared in the laboratory has been practiced for a considerable number of years in several cane-producing countries. To determine the value for Puerto Rico, under local conditions, of adopting this practice of artificially rearing *Trichogramma* wasps and releasing them in borer-infested fields, an investigation was started in the late summer of 1936 by the Division of Entomology of the Agricultural Experiment Station at Río Piedras.

#### HISTORY OF THE INVESTIGATION

For the first year, it was planned to make a preliminary survey of

the conditions as they occur naturally and normally, before any releases of *Trichogramma* were made, and with this as a basis, to commence releases in the second year. The reason for this preliminary survey was to determine if the cycle of host egg abundance and parasitism was the same in Puerto Rico as had been reported in other countries, to properly time the releases in the second year as to season, or time of year, or rainfall, or whatever factor might appear to be of most influence. In retrospect it appears that conditions observed in the first year were hardly typical in some regions, thus the releases made in the second year were successful (to the extent that they were successful) only by accident. Obviously, further field observations on natural conditions were essential, and no releases were made during the third year, but more time was devoted to the problem, with a reduction in the time period between observations. The problem had not been solved at the end of the third year, but enough data had accumulated to plan releases with some degree of certainty in the fourth year and to confirm them in the fifth year, while observations on natural conditions were continued during the entire period until field work was discontinued late in 1941.

The results reported in the present paper are for five years of continuous observations on the abundance or scarcity of the egg-clusters of *Diatraea saccharalis*, and on natural parasitism by *Trichogramma minutum*, in most of the important cane-growing regions of Puerto Rico. The results of the releases of artificially reared *Trichogramma* have been presented elsewhere, and do not affect these observations on natural parasitism. At the localities where releases were being made, only the check fields are here noted. As releases were usually repeated each week for a series of weeks in a region, however, the records of normal conditions at these points are available at shorter periods than elsewhere.

#### REGIONS

As originally planned, the observations were to be made for one week in the region between San Juan and Arecibo, for another week in the Isabela region, a third week in the Guánica to Ponce region, a fourth along the south coast east of Ponce, and a final week in the northeastern corner of the Island. (Because of the foreign parasite introduction work being conducted at the Federal Experiment Station at Mayagüez, many of the releases of which, it was thought, might be made in the cane regions adjacent to Mayagüez and thus affect the moth borer in that region, no observations were made at Rincón, Añasco, Mayaguez and Hormigue-

ros, and very few at San Germán.) As the investigation developed, it became clear that these regional units were too large, while individual records showed too much variation to be considered separately. Scattered observations anywhere in the region were eliminated, as were those far distant from a point where a co-operative observer of the Weather Bureau recorded rainfall and temperature. Eventually, observations were restricted to the localities given in the accompanying table (Table No. 1), which are arranged beginning at the southwestern corner of the Island, proceeding thence along the south, east and north coasts to the northwestern corner. That a clearer mental picture may be obtained of these regions, the average annual rainfall for the period September 1936 to August 1941, inclusive, and the maximum and minimum temperatures recorded at any time during this period are given for all localities for which records are available. It should be understood that these are extreme temperatures for the five years, not normal for the greater part of the time.

TABLE NO. 1

*Coastal Cane-Growing Regions of Puerto Rico*

AVERAGE ANNUAL RAINFALL	LOCALITY	TEMPERATURES MAXIMUM AND MINIMUM	
33.43 in	Guánica to Yauco	92°F.	53°F.
		(after December 1939)	
29.02 in.	Guayanilla and Tallaboa		
35.51 in.	Ponce	98°F.	57°F.
32.77 in.	Santa Isabel and Juana Díaz	93°F.	62°F.
		(after February 1940)	
38.59 in.	Salinas and Aguirre	96°F.	60°F.
56.33 in.	Guayama and Arroyo	96°F.	60°F.
89.21 in.	Patillas, Maunabo and Yabucoa	99°F.	61°F.
87.40 in.	Humacao and Naguabo	95°F.	54°F.
61.80 in.	Ceiba, Fajardo, Luquillo and Mameyes	93°F.	61°F.
75.65 in.	Río Grande, Loíza and Canóvanas	95°F.	56°F.
65.25 in.	Toa Baja and Dorado	99°F.	61°F.
66.68 in.	Manatí	98°F.	57°F.
54.29 in.	Arecibo	96°F.	58°F.
56.23 in.	Quebradillas		
66.06 in.	Isabela	94°F.	59°F.
83.59 in.	Coloso	98°F.	58°F.

The grouping into regions on the north coast was comparatively simple, with fields on each river valley naturally grouped together. Indeed, all field observations in the entire northwestern corner of Puerto Rico are so nearly the same at one time that, despite their being divided under the headings of Coloso, Isabela, Quebradillas, Arecibo, (and in some years, Manatí, Dorado and Toa Baja), for practical purposes they may be considered as forming one large region. Despite the diversity of soil, elevation, contour and rainfall, *Diatraea* and *Trichogramma* appear to find conditions essentially similar in this geographically diversified region, which constitutes possibly a fifth of the cane-growing area of the Island.

Just the opposite condition occurs on the south coast, which in soil, elevation, contour and rainfall from Ponce to Guayama is remarkably uniform. It would appear to be but a single environment, with practically unbroken cane fields, all under irrigation, extending in a narrow belt along the coast. From the standpoint of *Diatraea* and *Trichogramma*, however, there is no uniformity. Here occur the most tremendous variations in abundance, most often affecting all fields in a region, but sometimes only individual fields or those of a restricted area. Temperature and rainfall in the Santa Isabel and Juana Díaz region are so similar to that of Ponce on the west and Salinas on the east, with no natural geographic break or division, that the apparently arbitrary one here made is adopted solely because of the reactions of the insects as shown by our observations.

#### SELECTION OF FIELDS

Under optimum conditions, a locality record consists of the totals of from five to eight (or more) typical fields, non-adjacent, but not too far distant from the point at which the rainfall and temperature records are made. In the latter years of the investigation, such locality records were the rule, and were often made at three week intervals, but earlier, such optimum conditions were only approximated at some localities, and the time interval between records was four or five weeks, and sometimes longer. When an abundance of fields was available for inspection, the results as reported might to some extent depend upon the particular fields selected for observation. Since the purpose of the investigation was primarily to determine the abundance of *Trichogramma*, what seemed to us to be the least desirable fields were not inspected if better fields were available. Whether our standards were the same as those of the insects is a question which the available evidence does not answer. When no choice of fields was possible, the field which seemed least desirable to us rarely had as

many egg-clusters as the others, and often had none. On the contrary, when many fields were available, often one would be far below the average, and an additional half hour spent in examination (on the possibility that the piece selected for examination was a sub-normal sample) failed to materially change the result. Such sub-normal fields must be included in the records for a region, even if no egg-clusters were found. Quite often, however, if one apparently optimum field (by our standards) had few or no egg-clusters, that would prove to be the rule for the region at that time. Averaging such apparently negative results was held to indicate the culmination of unfavorable conditions for *Diatraea* oviposition, and to present just as true a picture as the average of fields with an abundance of egg-clusters at some other time.

For the most part, the fields examined were those most readily available along the main highway. Thus in some cases, the region as examined was miles long and only a few fields wide, and was of more logical shape only when parallel roads, or a triangle of roads, made the examination of a greater width possible. Large fields were preferred for inspection, and usually were examined clear across from one side to the other at about the middle. If the rows ran parallel to the road, the observer went part way up one side to avoid the end rows, which are often sub-normal if shaded by roadside trees. When a choice was possible, fields of uneven contour were avoided, as earlier examinations of such fields had sometimes shown marked concentration of egg-masses, as at the brow of a hill in humid regions, or in a swampy part, or along a small permanent stream in xerophytic regions. Comparable uneven distribution of egg-masses might occur in certain parts of a field apparently no different from any other part of the field, but more often the collection made crossing the field approximated that made on the return, and sometimes was exactly the same.

#### PERSONNEL

For making the field observations, the junior writer was employed specifically and exclusively, while the senior writer accompanied him when possible. Thus the bulk of the notes for the first part of the investigation is the joint product of their observations. Later, to reduce the time between observations, the work was divided so that the junior writer made most of the observations on the south coast, the senior writer those on the north coast, and those of the east coast and in the Isabela region usually made jointly. While the egg-counts of individual fields as thus



made are sometimes subject to wide variation from other fields of the region on the same day, or from previous or subsequent observations in the same field, it is believed that such deviations actually represent conditions as they were in the region at the time, subject to minor error in the selection of the sample, and are not due to the personal, subjective difference of the observers. Of course the personal element can not be entirely ignored, but when both observers were collecting in the same field, wide variations in their results only occurred when egg-clusters were scarce and what each one found was largely a matter of chance. When abundance of egg-clusters largely eliminated the factor of chance, collections were nearly identical. It should also be noted that what looks like a major variation when reported of only a few egg-clusters (due to chance, or personality of the observer, or selection of the field, or the rows in the field) really matters very little. When eggs are abundant, no such major error is likely to occur, and from a statistical standpoint, can not occur.

#### MAN - HOURS

After the first few experimental months, most of the observations were of only one man-hour, altho at first an earnest effort had been made to find at least ten egg-clusters before leaving the field. Theoretically, the use such a subjective unit as the man-hour may appear undesirable, yet experience indicates that this is the most useful and practical for this particular problem. To be sure, from it one can not calculate the number of eggs per acre, for the ease of walking thru fields with such different systems of cultivation as are used in Puerto Rico and of different heights of cane and varied weather conditions, varies considerably. Even less possible is any estimate of the number of egg-clusters for the area examined, for, except for those indicated by chlorotic spots, half of the egg-clusters, being on the other side of the leaf, can not possibly be seen, and some also escape observation because of the wind shaking the leaves, or sunlight reflected from them, even tho they are apparently in plain sight. The man-hour merely indicates the number of egg-masses collected by one man in one hour walking naturally thru the field and collecting those which can be found without stopping to turn the leaves, or to examine the plants with extreme care.

#### OTHER SPOTS

In searching for the egg-clusters of *Diatraea saccharalis*, the observer must look for a small object of indeterminate shape and size; of a color

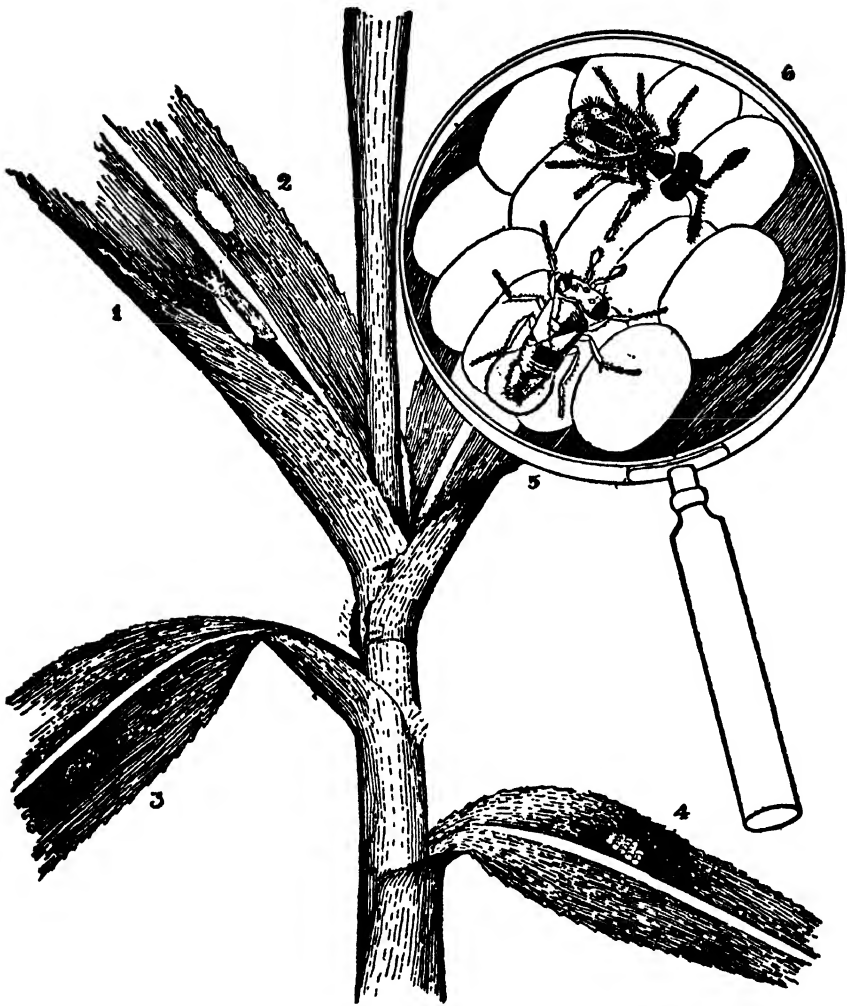


Fig 1 Shoot of sugar-cane, on the leaf of which is resting. (1) an adult of the moth borer, *Diatraea saccharalis* F., and to which is attached (2) a fresh cluster of *Diatraea* eggs, (3) an egg-cluster which has been eaten by ants, and is as hard to represent in a drawing as it is to see in the field, (4) a partly parasitized egg-cluster and, greatly magnified under the lens, a fresh egg-cluster on which is resting (5) an adult of *Trichogramma minutum* Riley and (6) an adult of *Prophanurus alecto* Crawford. (Original, drawn by G. N. Wolcott).

which may be creamy white (eggs just laid), light yellow (a few days later), spotted with orange or red (next to last day before hatching), opalescent grey (recently parasitized), blue-black (parasitized), mottled grey (parasites emerged) and promptly differentiate it from other spots of similar shapes, sizes and colors. Furthermore, if the egg-cluster has been eaten by ants, often the lower layer of egg-shell that attached the eggs to the leaf is all that remains, and sometimes no more than the merest ring of chorion outlining the original shape of the cluster. (Fig. 1).

The mere presence of a moth-borer egg-mass on the cane leaf also sometimes (in reality, not very often) causes the eventual appearance underneath or above it of a chlorotic spot in the leaf-tissue, which of course is also visible on the other side of the leaf. The spot itself remains after the egg-cluster has fallen off normally, but in the present investigation, no chlorotic spot was counted unless some bit of the egg-shell remained to prove that an egg-mass had previously rested there. Nevertheless, all chlorotic spots of the shape and size of a *Diatraea* egg-cluster had to be examined, on both sides of the leaf.

Besides chlorotic spots which might be due to other causes than the actual or previous presence on the leaf of an egg-cluster, the observer must also promptly differentiate and mentally discard the following things or spots on cane leaves: split places in the leaf, or holes eaten into or thru the leaf, or the shadow of such a hole on the leaf below; lizard excrement or bird excrement, or mistletoe seeds, or other seeds of fruit scattered by birds; black shiny spots of what looks like crank-case oil, thick black hard shiny masses, granular white, yellow orange, brown or black masses; individual dew or raindrops (which look like "eaten" egg clusters); mechanical abrasions and points where the midrib has been broken; fertilizer and soot from the mill; fungus leaf spots; masses of spider silk; fresh or hatched egg-masses of *Laphygma frugiperda* S. & A.; hatched egg-masses of *Diaprepes abbreviatus* L., which often turn the leaf-tissue underneath reddish brown; egg-masses of the leafhopper *Kolla similis* Walker, which are light green when first laid in the leaf-tissue, often becoming reddish brown later; egg-masses of the Fulgorid, *Saccharosydne saccharivora* Westwood, which are often yellow or brown on the other side of the cane leaf or midrib in which they are deposited; adults of the Fulgorid, *Oliarus franciscanus* Stal, which are grey, and adults of the Ortalid fly, *Euresta thomae* Loew, which are barred with shining black and white. To be sure, many of the mentioned spots or objects may seem to be most improbable of confusion with *Diatraea* egg-masses, and in most cases they

are immediately recognized for what they are, but each such spot or object must be noted and promptly identified before the attention can be re-directed in further search.

#### SUMMARIZATION OF DATA

At the conclusion of the field work, copies of the field notes were made on smaller cards, measuring two by three inches, which could be readily arranged and re-arranged without disturbing the chronological arrangement of the numbered field notes retained in their booklets of fifty for reference in the files. From these little cards, shuffled and re-arranged as one desired according to any particular factor, or group of factors, it was possible, without danger of missing any records, to obtain exact data in summarized form on fields, man-hours, percentage of parasitism and on each class of egg-clusters by month, locality, plant or ratoon, trash burned of ratoon cane or not burned, and on height of cane.

#### CLASSIFICATION

All egg-clusters collected were retained at least for careful re-examination when recording the data at the end of the inspection. For recording, they were grouped under six headings: fresh, eaten by ants, hatched, parasitized, partly parasitized and parasites emerged. These groupings seem sufficiently definite, but a small number of egg-clusters was collected which could not be thus readily classified, part of the egg-cluster assigning it to one group, the other part to another. Of course it was possible to note the exact condition of such egg-clusters, but this only complicates the final consolidation of data. In practise, one placed the egg-cluster in question in one classification, and noted its possible inclusion in the other. Fortunately, the number of such egg-clusters was so small that it could be ignored without materially affecting the final results. The total number of egg-clusters collected in five years from the 4,595 fields (not including those in which releases were made, and twelve at scattered locations) was 45,430, or an average of almost ten per man-hour.

#### "EMERGED"

As used for these records, the heading "emerged" indicates that parasites had emerged from the egg-cluster previous to its collection, and not caterpillars. 5,391 egg-clusters were collected from which parasites had emerged, or 12% of the total number, and slightly less than half as many

as were found parasitized. The finding of many emerged egg-clusters in a field or a region should indicate the close approach of the end of the cycle of parasitism which started with an abundance of fresh egg-clusters, culminated in an abundance of parasitized egg-clusters, and ends with a preponderance of emerged egg-clusters. This is true in general, and would be more obvious if such egg-clusters had better adhesion and did not drop off, or disappear out of sight under fresh leaves as soon as their mission is accomplished. The ratio between 5,391 emerged and 11,433 parasitized presumably indicates the length of time that the egg-masses remain attached to the leaves after the parasites have emerged, expressed in terms of the period from the appearance of parasitism to emergence of the wasps. Actually, one finds emerged egg-clusters in some fields long before the cycle is that far along in other fields of the same region, thus the graph drawn for the averages of a region may show many emerged egg-clusters while parasitism is still increasing. In practice, the number of emerged egg-clusters is added to that of partly parasitized and parasitized to obtain total apparent parasitism.

#### "PARTLY PARASITISED"

The term "partly parasitized" as here used indicates that more than two eggs in the clusters had been attacked by *Trichogramma*, but that the remainder were fresh, had hatched, or had been eaten by ants. If only one or two eggs had not been parasitized, the egg-cluster was considered so nearly entirely parasitized as to be included under that heading, while if only one or two eggs in the cluster had been parasitized, this small amount of parasitism was ignored, the two conditions thus canceling each other out so far as our records are concerned. It is presumed that most of the failure of *Trichogramma* to parasitize all the eggs in a cluster is due to cold weather, even the minimum temperatures in the middle of the day in Puerto Rico are rarely low enough to affect its activities. At many localities, most of the partly parasitized egg-clusters were found during the cooler months of the year, especially on plant cane, but with numerous exceptions. The totals for all localities show four times as many in December as in June and July, and five times as many as in August or September, but with differences between other months less pronounced. Actually, the grouping has little importance in Puerto Rico, the recorded total of 638 being only about 4% of all parasitized, or 1.2% of all egg-clusters. At no locality was 3% of the total number of egg-clusters thus partly parasitized, and nowhere on the south coast more

than 1%. Indeed, the figures for partly parasitized are so small that when the consolidated data for a locality were being represented in a graph, they were combined with emerged to be seen at all.

“PARASITIZED”

The time-lag after *Diatraea* eggs are parasitized by *Trichogramma* wasps before they begin to show the effect of parasitism by turning black is between one and two days. As used for these records, the term “parasitized” is only for those egg-clusters unquestionably attacked as indicated by at least the beginning of change in color. Somewhat inconsistently, however, all egg-clusters collected while they were being attacked were also counted as “parasitized”, even tho there would be no indication of this for a day or two, once the wasps had flown away. By this definition, the number of parasitized egg-clusters collected was 11,433, or 25% of the total of all egg-clusters. To obtain the figure of total parasitism, to this is added the 638 of partly parasitized and 5,391 emerged, which gives 17,462, or 38% of all egg-clusters.

Of all the axioms of parasitism, the one most universally assumed is that abundance of the host implies subsequent abundance of the parasite, either immediately or closely following. The egg stage of *Diatraea* and the entire life-cycle of *Trichogramma* each occupy such a very short time that one would expect abundance of egg-clusters to practically coincide with percentage of parasitism. Of all the results obtained in this investigation, no other set of figures is so uniformly consistent as that giving for each locality the percentages of parasitism and abundance of egg-clusters. Even where, as at some north coast localities, the percentage of parasitism began at 25% or 26% when only one or two egg-clusters were collected per man-hour, the next higher division of from 3 to 6 egg-clusters per man-hour showed some increase in parasitism, going up rapidly to the classes of greatest abundance: 31 to 62, and 63 and over, with parasitisms of 66% and 73%. At Santa Isabel, starting with no parasitism in the 1 to 2 egg-cluster class, parasitism goes up rapidly and reaches 68% in the 63 egg-cluster or over class. At some localities, parasitism goes up more slowly, as at Guánica, starting at 5% and ending at 33%, but in every case the trend is consistently upward paralleling the increasing number of egg-clusters. Averaging all localities: 1 to 2 egg-clusters are 13% parasitized; 3 to 6 are 19% parasitized; 7 to 14, 30% parasitized; 15 to 30, 42%, and 31 to 62 and 63 and over both 53%. It should be especially noted that these are average figures, smoothing

over many exceptions, but that the averages are so consistent would indicate the truth of the axiom for this particular instance of parasitism.

"EATEN BY ANTS"

The heading "eaten" was originally intended to include all egg-clusters in any stage of development which had been fed upon by any predator. Especially in those cases where part of the eggs were still uninjured, the status of those remaining was of more importance for the purpose of this investigation for including under the heading of parasitized or fresh than the fact that part of them had been eaten. Empty egg-shells were also sometimes eaten by ants, and in such cases, the fact that the caterpillars had hatched out of the egg-cluster was of greater importance for this investigation than that the egg-cluster had later been disturbed by ants. In all of these cases, the egg-cluster in question was invariably listed under the other heading, rather than as "eaten". Indeed, no space for eaten was provided in the first provisional card blanks, but the common occurrence of such egg-clusters required that a space be made in the re-designed card blanks used after the first months.

Despite the wide variation in the character of "eaten" egg-clusters, however, varying from complete consumption of everything except the rim around the edge of the cluster, making any assumption as to the original character of the cluster impossible, to partial consumption of some fresh or parasitized eggs, or merely messing up of empty egg-shells, it is believed that all of these cases are due to a single cause. A small black ant, *Monomorium carbonarium ebeninum* Forel, as determined by Dr. M. R. Smith, has repeatedly been found in the field feeding on both fresh and parasitized egg-clusters, as well as on the egg-shells of hatched and emerged egg-clusters. No other ant, or other insect or small animal has been found in Puerto Rico feeding on *Diatraea* egg-clusters. As nearly one-sixth of all egg-clusters collected are recorded as having been eaten, *Monomorium ebeninum* would be a factor of very considerable importance in the control of the moth borer if it were more discriminating in its choice of the character of eggs eaten, and avoided those parasitized by *Trichogramma*. In many instances, however, when *Trichogramma* is not present at all, or is very scarce, one gets the impression that the ants eat more of the egg-masses than when the parasite is present in abundance. This is especially marked at Guánica and Ponce, but sometimes the reverse is true elsewhere. It is so often true, however, that this ant may be considered as largely beneficial in aiding in the control of *Diatraea*. A total of 7,377 egg-clusters

completely eaten by ants is recorded or 7,724 counting those partly eaten, and those also counted under another heading, but not empty egg-shells bitten into and messed up. This is 17% of all egg-clusters collected.

At Guánica this little black ant is an especially important factor in natural control of *Diatraea*, for 23% of all egg-clusters had been eaten by ants here, as compared with 27% total apparent parasitism by *Trichogramma*. Ants were of least importance in the northwestern corner of the Island, and at Santa Isabel on the south coast, accounting for only 10% of all egg-clusters at these points; elsewhere they ate from a fifth to an eighth.

#### "HATCHED"

The heading "hatched" is used for indicating egg-clusters from which caterpillars hatched, as opposed to those from which parasites emerged. The original purpose in including the records of hatched and emerged was to obtain a cross-section of conditions as they were a week ago, as well as those at the time of inspection. Actually, all that is possible is to obtain a cross-section of present conditions, for the data on hatched and emerged egg-clusters are not comparable. *Trichogramma* requires approximately twice as long for its development to adult in the host egg as does the embryo of the moth borer for its development to caterpillar. Thus, some parasitized eggs are of the same age as some hatched egg-clusters, and there is no way of judging the age of egg-clusters from which caterpillars have hatched. Nevertheless, the data on hatched egg-clusters are most important as indicating beyond a question of doubt how many egg-clusters actually did escape natural control, even if the length of the period during which they remain attached to the leaves is of uncertain duration. A total of 12,635 hatched egg-clusters was collected in five years, or 28% of all found.

In plant cane, the number of egg-clusters found hatched varied from somewhat over two and a half egg-clusters per man-hour at most points on the north coast, thru nearly four per man-hour from Ponce to Guánica and Loíza to Humacao, with a high of over six egg-clusters per man-hour at Salinas, Guayama and Yabucoa. The extremes are even more marked in ratoon cane: one and a half egg-clusters per man-hour on the north coast, around four or over from Guayanilla to Yabucoa, with an outstanding high of 8.2 egg-clusters at Salinas. As will later be explained, to show how large is the number of eggs escaping natural control, to this also must be added a part of the apparently fresh egg-clusters, the part



naturally being largest where most hatched egg-clusters are found, and smallest where natural control is most effective and most fresh egg-clusters become parasitized or are eaten by ants.

The apparently negative evidence of finding only a few hatched egg-clusters in a field is very definite proof of how unfavorable conditions had been in that field for the days and weeks immediately preceeding for *Diatraea* oviposition and *Trichogramma* parasitization. Finding an abundance of only hatched egg-clusters just as surely indicates a period of a few days, a week or more ago, most favorable for *Diatraea* oviposition, but developing so rapidly that control by *Trichogramma* could not take place. The first condition is common everywhere on the Island; the second is less often noted, being too late a discovery of conditions that did offer ideal conditions for the release of laboratory-reared parasites a few days previously. How to find such fields in time to take advantage of the opportunity presented, or rather, how to predict their occurrence some time in advance, is the key to successful *Trichogramma* liberations.

#### "FRESH"

The heading "fresh" is merely the shortest term under which to group all the egg-clusters which were unhatched at the time of collection, and were apparently unparasitized. It includes all egg-clusters varying in freshness and depth of coloration from the light cream to those plainly showing the curled-up caterpillar within. Thus the actual time difference between an egg-cluster listed as fresh and one entered as hatched may be a matter of a day, or only a few hours. This seems somewhat arbitrary, but in reality is vital, for the hatching of the egg-cluster eliminates the possibility of its being parasitized or eaten by ants. For this investigation, all those eggs which were in fact parasitized, but showed no sign of it at the time of collection, were also listed as "fresh".

It must be remembered, therefore, in interpreting the percentages of parasitism as given, that parasitism, if it occurred at all, was presumably somewhat higher than indicated, and might be very considerably more. For some months, all the so-called fresh egg-clusters were retained until their true status was obvious, and from a third to nearly two-thirds were found to be parasitized in fact. As the bulk of the fresh eggs retained were from fields where the observed parasitism was low, it would indicate nearly total parasitism of the smaller number of apparently fresh eggs collected in fields where parasitism was high. To this must be added the parasitism which would have resulted in the actually fresh

egg-clusters at the time of collection had they remained undisturbed in the field, which naturally is also a greater chance of being parasitized where most of the egg-clusters are also parasitized and the parasites are abundant. Constructing a somewhat arbitrary table with allowance for both of these factors, no increase in actual parasitism is anticipated if observed parasitism is 30% or less; 50% increase in parasitism is presumed due to these two factors if observed parasitism is 50%; total parasitism if observed parasitism is 70% or more. Possibly some correction should be made for the effect of abundance of egg-clusters on parasitism, also for natural control by ants if the clusters had been left in the field, but this makes the table so complicated as to be unworkable in practise.

The total number of fresh egg-clusters was 7,956, or 18% of the total. Had they not been collected, three possible fates awaited these apparently fresh egg-clusters: (1) they might escape all perils and hatch normally, (2) they might be eaten by ants, or (3) they might be parasitized by *Trichogramma*. These three possibilities are not probable in exactly the proportion that the egg-clusters collected by us actually did suffer these fates. As Dr. G. W. Kenrick, Professor of Physics at the University of Puerto Rico, points out: consumption by ants or parasitization by wasps might happen at any time after they were laid and during most or all of the incubation period following, while hatching could occur only at the end of these days of peril. It is apparent, therefore, that many parasitized or eaten by ants have already been subtracted from those recorded under the heading "fresh", and none under the heading "hatched", thus a proportionately greater number of them will hatch. To obtain a really exact answer requires more exact biological data than is available, but using various approximations from the total data figures reported for the whole period, Dr. Kenrick, solving the problem by two very different approaches, by either obtains approximately the same answer. He finds that the number of fresh eggs which will survive to hatch if left in the field is somewhat more than half of those collected, rather than the one-third indicated by the percentage noted of the actual collections. There is only an apparent conflict here with the high probability of fresh eggs becoming parasitized when numerous in fields where some or most other eggs are parasitized, for such cases occur comparatively rarely. Dividing up the "fresh" eggs according to their presumed fate and adding it to those actually collected indicates that 38% of the eggs actually do hatch, and by difference, a total destruction due entirely to natural causes of 62%. That is, for Puerto Rico as a whole, for all seasons and times of year,

at all localities and without distinction as to whether the cane is plant or ratoon, nearly two-thirds of all *Diatraea* eggs are destroyed naturally, without any effort on the part of the grower to produce such a condition.

#### HEIGHT OF CANE

That the data as collected might be comparable in as many factors as possible, all observations were made in cane of a height that could be readily examined as one walked thru the field. In practice, this meant that cane less than a foot high was normally not considered worth the time necessary for examination. A few weeks later, the cane is of just the right height for easiest examination: eighteen inches to two feet. Ordinarily, if a field is examined once, the tendency was to continue examinations in the same field as long as such were possible. Often one, and sometimes two following examinations were made, and during periods when growth was slow, or when fields were being used as checks on others in which releases had been made, this might mean four or five consecutive examinations in the same field in one crop season, as well as examination in preceding and succeeding years. In some cases, considerable differences appeared in the number and character of the eggs found in successive examinations, suggesting that the difference might be due to the height of the cane. However, when a greater number of data of this character were available, it at once became apparent that the outstanding differences in different fields canceled out. Indeed, when all the data for each locality for each month are arranged according to height of cane, the result is surprising uniformity at all heights. Consolidating all data from all localities eliminates slight inequalities due to inadequacy of number of observations at the extreme heights. All the variations from normal in the final consolidation are within the limits of statistical error, and not only for the total number of egg-clusters, but also for each kind of egg-cluster: fresh, hatched, eaten by ants, parasitized and emerged.

The ants climb the higher cane to eat eggs just as readily, and *Trichogramma* recognizes no difference between high and low cane. Thus one may state without reservations that between eighteen inches and four feet in height, the height of the cane has no effect on the number and character of *Diatraea* egg-clusters present on the leaves. This is of the very greatest importance in this investigation, for it at once eliminates one possible cause of variation that one might have to consider in explaining results. The statement is limited to the height of the cane examined, but presumably it applies to all cane of whatever height. As regards oviposi-

tion by *Diatraea* moths, and parasitism by *Trichogramma*, the canopy of cane leaves would seem to be the same whether it is two feet or twenty feet above the surface of the ground.

Concentrating observations on cane from eighteen inches to three or four feet in height is quite different from the practise of other investigators working on the same problem in other countries, who have followed the same cane thru the season until it was harvested. No other course was possible in some of these other countries, but since cane of practically all heights is present during every month of the year in Puerto Rico, it seemed desirable to take advantage of this condition and eliminate the differences which might be due to the mechanical difficulties of making observations in high cane.

#### VARIETY OF CANE

The variety of cane has a very definite effect on the amount of borer injury to the stalk, as the varieties with the hardest rind and the most cellulose or fiber are so discouraging to caterpillars attempting to eat them that fewer survive than in the softer, sweeter canes. So far as oviposition by moths is concerned, this factor does not operate, or, if it occurs, is obscured by other factors. The variety B H (10) 12 is so generally grown on the south, POJ 2878 on the north coast, with scattered fields of M 28 in the northwest, and Fajardo seedlings in the northeast as to give little scope for comparison. In the one or two variety experiments examined, no apparent difference in oviposition was noted, but the paucity of comparable data makes a conclusive statement impossible.

#### ENVIRONMENT

In addition to spaces for noting the character and number of the egg-clusters, their total and the percentage of parasitism, the field note blank forms as finally designed for use in recording the presumably pertinent data in this *Diatraea* egg-cluster survey, also had spaces for the number of the record, the numbers of previous and subsequent observations in the same field, the date and exact time of day, the man-hours, the locality, the name of the owner of the field or the name of the Hacienda and the piece or tablón number, and its exact location (if on the main road) by the kilometer-hectometer post, whether the cane was plant or ratoon, and, if the latter, if the trash had been burned or not, the height or age of the cane at the time of observation, the soil and contour, the

rainfall (annual, for the previous week and for each of the preceeding six weeks), and the environment on all four sides: north, east, south and west.

The extensive notes on environment were primarily for noting any possible effect on abundance of egg-clusters. Incidentally, it was of the greatest value in noting on which side of the main road was the field examined, which, together with the kilometer-hectometer post, enabled one to identify the field exactly, and later correlate with observations in previous or subsequent years of the same field. Subtracting such incidental notes as those of "main road", "dirt road", "cane road", and "railroad", which can hardly have any effect on borer eggs, leaves 20,587 items in the environment of the total of 4,707 fields (including those in which releases were made, and a few at localities later eliminated) which were examined. Obviously, some fields had more than one factor on one or more sides, but this was the exception. Aside from the factor of "main road", one might think of the factors of environment as here given as possibly describing the typical Puerto Rico cane field, altho practically all of the fields were selected for suitability for examination and availability from the main road, and, to this extent at least, may not have been typical or average.

The factors of the environment as recorded for 4,707 cane fields in Puerto Rico observed during five years, are as follows:

high cane	8,372	houses	981
cane being cut	502	town	154
young ratoon	3,370	cemetery	37
young plant cane	939	cane-hoist	39
abandoned cane	136	Central	45
<hr/>		stables	19
sugar-cane	13,319	dipping vat	20
		bridge	17
		<hr/>	
malojillo meadow	13	structures	1,312
corn	85		
<hr/>			
other <i>Diatraea</i> hosts	98	pasture	2,797
"		plowed land	463
bananas	63	airport	22
pineapples	19	batéy	20
tobacco	33	ocean	79

cotton	15	creek or river	550
sweet potatoes	21	reservoir or lake	82
minor crops	143		
pigeon peas	19	geographic	4,013
cowpeas	13		
alfalfa	4	main road	4,347
		dirt road	361
crops	330	cane road	796
		railroad	691
coconuts	137		
grapefruit	48	roads	6,195
coffee	9		
trees	408	TOTALS	
casuarinas	8	sugar-cane	13,319
windbreak	28	other <i>Diatraea</i> hosts	98
mangrove swamp	78	crops	330
caña brava	31	trees	1,525
forested mogote hills	777	structures	1,312
		geographic	4,013
trees	1,525	roads	6,195
			26,792

Eliminating from consideration the various kinds of roads, nearly half of the environment was high cane, and sugar-cane in some stage of growth was almost exactly two-thirds of all environments. Obviously, the isolated cane field is exceptional, and only a single instance was noted of the isolation of the fields affecting the number of borer eggs. A field of young plant cane between Manatí and Vega Baja, surrounded on all sides by pasture, or forest and brush-covered mogote hills, had practically no egg-clusters the first year that it was examined, yet in the following year as ratoon cane, when time had allowed some chance for infestation, the number of egg-clusters was comparable to other fields in the same region. This shows the temporary advantage of seed selection, and also how temporary is such an advantage in Puerto Rico, where isolation from other fields is so difficult. Fields with the Atlantic Ocean or mangrove swamp to the north and east (from which the prevailing winds blow on the north coast) showed no advantage over those with cane on all sides, altho possibly they might have done so when plant cane. On the

other hand, fields with just cut cane or young ratoon cane to the leeward in no case had a noticeably greater abundance of eggs, due to moths flying out of the cane recently cut to oviposit in the young cane to the windward, than the average of others in the same region. Since we were anticipating such a difference, and looking for it in every case where these special conditions of environment occurred, that no such difference was found would indicate that every instance is a special case, and the result might not be due to the anticipated factor. Indeed, except in a very few individual cases, the data on environment would seem to be interesting rather than significant. A careful and detailed statistical treatment might indicate results not anticipated, but it must be remembered that the people actually making the observations saw no such possible clue when in the field.

#### OTHER PARASITES

The environment of the cane field in Puerto Rico plays no role comparable to that reported in some other countries, of serving as a reservoir of hibernating eggs of other kinds of moths and butterflies for *Trichogramma* during the winter. Indeed, as sugar-cane is generally grown in Puerto Rico, in large solid blocks, usually carefully weeded and with a minimum of other plants on which the eggs of other moths and butterflies might be laid, *Trichogramma* is in fact largely restricted to the eggs of *Diatraea saccharalis*. Not only that, but another dark race of *Trichogramma* is also present in cane fields, attacking the eggs of the Hesperiid butterflies, *Prenea nero* F. and *Prenea ares* Felder, but according to our observations, not those of the Crambid moth, *Diatraea saccharalis*. This dark race of *Trichogramma* is not to be confused with another black egg-parasite of the sugar-cane *Prenea* an Encyrtid which Mr. A. B. Gahan identifies as a species of *Ooencyrtus*.

Nor should this dark *Trichogramma* be confused with the black Scelionid wasp, *Prophanurus alecto* Crawford, first reared in Puerto Rico from the eggs of *Diatraea saccharalis* at Río Piedras and Toa Baja in 1921 (Wolcott 1922). Until 1938, this parasite was not again found in Puerto Rico (Wolcott 1939), but in October of that year it was the only parasite attacking egg-clusters of *Diatraea* at Isabela (determination confirmed by Mr. C. F. W. Muesebeck) and mingled with *Trichogramma* at Quebradillas and Coloso. A month later it had disappeared in the Isabela region, but was responsible for seventy or eighty percent parasitism of *Diatraea* eggs at Guánica (and thoroly disorganizing the *Trichogramma*

release experiment there), where it had never before been collected. By the next month it was gone at Guánica, but had appeared at Patillas and Arroyo, where it had disappeared by the next observation. It was very abundant at Las Piedras in April 1939, and noted at Isabela, and again in December at Quebradillas, but has not since been recorded from anywhere in Puerto Rico.

#### PLANT OR RATOON

One of the most obvious and really fundamental differences between the various fields of a region is that some are of plant cane and others are ratoon. On the assumption that this might have an effect on oviposition by *Diatraea*, we were constantly on the look-out for such a difference between plant and ratoon fields. Yet rarely did we find a pair of truly comparable fields showing an apparent effect in one way that it was not promptly duplicated by a similar pair showing the supposed effect in the other direction. Averaging a sufficiently large number of fields, however, such as all those examined in the same month for five years at one locality, or all of those from one locality, or all those for one month, gives a very definite but not very consistent difference. Ratoon fields at Coloso, Isabela and Arecibo average twice as many egg-clusters as do fields of plant cane at these localities, and at Santa Isabel, Salinas, Dorado and Manatí, the difference is pronounced, (see Table No. 2). At other localities, the difference is not significant, and at Guayama, Yabucoa, Fajardo and Loíza there is no difference. Twice as many egg-clusters were found in ratoon fields in May and July, and almost twice as many in April and June, as in plant cane, but in December, February and March the collections averaged practically the same.

Parasitism of egg-clusters in ratoon cane averages double that in plant cane at Guánica, Ponce, Santa Isabel, Fajardo, Dorado and Manatí, and even more in all localities in the northwestern corner of the Island. The months of May and June also have ratoon cane with twice the parasitism of that found in plant cane, and with significant differences in all other months. The differences in abundance of egg-clusters and parasitism are all in the same direction, and en masse are unquestionably significant, even tho they rarely parallel each other at the same locality.

The total number of fields of plant cane examined was 2,343, which had 22,064 egg-clusters, or 11.5 per man-hour, and the percentage of parasitism was 27%. The total number of fields of ratoon cane in which the trash had not been burned was 2,144, which had 22,468 egg-clusters.



TABLE NO. 2

EGG - C U L T U R E S      P E R M A N - H O U R

LOCALITY	MAXIMA		AVERAGE TEN HIGHEST		AVERAGE		MINIMA		PERCENT PARASITISM	
	PLANT	RATOON	PLANT	RATOON	PLANT	RATOON	PLANT	RATOON	PLANT	RATOON
GUANICA	160	112	78	55	11.8	13.7	10 zeros	3 zeros	20%	39%
GUAYANILLA	36	36	28	28	9.3	12.	7 zeros	2 zeros	21%	33%
PONCE	88	88	57	58	12.5	14.7	4 zeros	2	23%	41%
SANTA ISABEL	90	140	65	88	17.4	27.3	2	1 zero	31%	63%
SALINAS	132	112	88	67	18.3	22.9	1	1	27%	34%
GUAYAMA	84	84	63	68	15.6	15.8	3 zeros	3 zeros	28%	42%
YABUCOA	96	172	50	68	16.3	16.	4 zeros	1 zero	33%	50%
HUMACAO	52	56	33	43	11.7	13.7	4 zeros	1 zero	30%	44%
FAJARDO	42	42	28	28	7.6	8.4	20 zeros	22 zeros	18%	32%
LOIZA	50	56	31	33	9.4	8.3	14 zeros	13 zeros	25%	37%
DORADO	70	114	37	63	9.	14.5	18 zeros	16 zeros	29%	52%
MANATI	93	136	44	79	10.	18.1	28 zeros	9 zeros	37%	68%
ARECIBO	48	66	24	45	7.1	14.2	8 zeros	8 zeros	24%	62%
QUEBRADILLAS	50	66	32	49	10.9	12.9	4 zeros	7 zeros	32%	65%
ISABELA	72	78	32	51	8.1	16.3	8 zeros	5 zeros	24%	65%
COLOSO	56	60	24	41	6.9	11.1	10 zeros	8 zeros	25%	67%

or 14.8 per man-hour, with a percentage of parasitism of 50%. While the total of fields examined in each division was substantially the same, most of the plant cane was examined in the fall and winter, with mostly ratoon in spring and summer, and if the season had any effect, this (especially in the northwestern corner of the Island) might also show up as an apparent difference between plant and ratoon.

#### BURNING OF TRASH

The burning of cane trash at the time the crop is harvested is generally practiced in most countries where cane is grown, but in Barbados and Puerto Rico it is the exception rather than the rule. Without attempting to evaluate its agricultural status, from the standpoint of natural control of insect pests the burning of cane trash can only be considered a calamity. When the non-burning of cane trash was first recommended for adoption in Puerto Rico, its effect only on moth borer and *Trichogramma* was considered, but now the destruction of toads, which rarely are able to escape from between cross-fires, is of even greater importance because of the role that they play in the control of white grubs.

The non-burning of cane trash has been so generally adopted in Puerto Rico (Wolcott 1933) that it is now difficult to find many fields in which the trash has been burned. No attempt to find such fields, or to avoid them, was made in the present investigation, and it is largely accidental that 110 fields out of the total of 4,597 were of this character. This is a rather small number from which to draw conclusions, but their average of 10.3 egg-clusters per man-hour and parasitism of 31% is so similar to that of plant cane, rather than to ratoon cane of which the trash had not been burned, as to suggest that the burning of cane trash actually did have a definite effect.

#### RAINFALL

As soon as data on the abundance and scarcity of *Diatraea* and *Trichogramma* had begun to accumulate, attempts were made at summarizing them so that trends could be observed and theories evolved and tested to determine what were the essential factors affecting these insects. On the assumption that rainfall would be found to be the decisive factor affecting the abundance of egg-clusters of *Diatraea*, as it unquestionably appears to be of larval injury to stalks (Wolcott 1915), primary attention was given to this factor.

In the first observations, the great variation in the number of egg-

clusters collected in the wet and the dry parts of the Island seemed to indicate that total annual rainfall for each locality in which the observation was made would give the key to the number of egg-masses to be expected in the fields of that region. Later observations indicated that this assumption was quite incorrect, and led to a re-designing of the record card so that a space would be available for recording the rainfall of each of the seven weeks preceeding the date of observation, as well as the annual rainfall for that locality. The monthly summary of the local station of the Weather Bureau, published as "Climatological Data, West Indies and Caribbean Service," gives daily records of the rainfall at all points where we made field observations, and much time and energy was devoted to consolidating these daily records into weekly summaries for our field notes. At that time it was impossible to predict how much of the rainfall data was essential, but it seemed preferable to include space for too much, rather than too little. Even at that time it was obvious that the total amount of rainfall in the week gave only a very rough approximation of what should be known as to the conditions of humidity as they affected the emergence and oviposition by the female moth, and parasitization by *Trichogramma*, but no closer approximation is possible for extensive field observations.

So far as the *Diatraea* moth is concerned, several observations had indicated that a field otherwise dry, and capable of growing cane only as it was irrigated, had quite a different character if a small permanent stream flowed thru it, for all egg-clusters were found close to the banks of the stream. Such a modification of the micro-climate of that part of the field was obviously of much greater local importance than rainfall, and lacking any definite theory as to how humidity affected the *Diatraea* moth and the parasite of its eggs, the time of day or night when the rainfall came, and its distribution thru the week might prove to be of more importance than its actual amount.

In re-designing the record card, the character of the soil, as it might affect the micro-climate close to the ground, was considered to be of possible importance. In only a few specialized cases did this appear to be so, as in the xerophytic part of the Island, heavy clay soils, especially the "pollal" or poorly-drained phase of such soil, maintain a microclimate supposedly more suitable for the insects. Contour may have an appreciable effect when it separates the level swampy field from a higher slope, even tho rainfall is the same for all parts of the field. For such specialized cases, soil and contour may affect the results, but for the great bulk of the fields,

all of our efforts to exactly locate the field on the soil survey map of the Island and record the name in our field notes appear to have been of little value.

The effort to obtain exact data on rainfall, and on soil and contour as it affects humidity in the field, is worth while only on the assumption that variations in tropical weather as normally experienced in Puerto Rico are vital to cane insects. Sugar-cane is extensively grown, for instance, where the annual rainfall in some years is less than 30 inches. During 1939, at Central San Francisco, Guayanilla, the total annual rainfall was 18.82 inches; at Hda. Florida, Santa Isabel it was 22.07 inches; at Central Aguirre it was 24.32 inches; at Ponce it was 26.70 inches; and at Hda. Santa Rita, Guánica it was 29.82 inches. Of course the commercial growing of sugar-cane under such conditions of rainfall is possible only when it is abundantly supplemented by irrigation. Indirectly, irrigation is essential to *Diatraea* in assuring an abundant supply of food for its larval stage, but that gives no clue as to the direct effect on oviposition, if any. Our observations proved to be of little value in this connection, for we repeatedly found an abundance of egg-clusters on young cane so dry that the leaves had begun to curl, not only on the south coast, but also during drought on the north coast. Quite as often, one found only a few old hatched egg-clusters on drought-curled leaves, indicating that no eggs had been laid within weeks. If either one or the other condition only had been found, some conclusion might have been reached. Special efforts, immediately and one and two days after heavy rains in such regions, to determine if rain after drought did have a pronounced effect on oviposition, were also inconclusive.

Sugar-cane is commercially grown at several points where the annual rainfall averages close to 90 inches. Despite the high total annual rainfall, such regions often experience periods of drought weeks and sometimes months in length, and if the fields are reasonably level, the rainfall is supplemented by irrigation. Naturally, these are the best fields, and as such were those which we usually examined in a region. The net result was that most of the fields examined anywhere in the Island were those which received sufficient water for optimum growth: either mostly heavy rainfall supplemented by irrigation, or light rainfall supplemented by heavy irrigation. Thus the tremendous difference in annual rainfall, from less than 30 inches to nearly 90 inches in a year, had little effect on growth of cane, and also appears to have had no consistent effect on oviposition by *Diatraea*, at least in most of the fields which we examined.

As a most extreme example, one might cite the 16.17 inches of rainfall in the week ending January 2nd, 1937 at Loíza, which apparently eliminated parasitism temporarily, and somewhat decreased the total number of host eggs, but with complete recovery of abundance of host eggs and parasitism by the time the fields in this region were next examined. Fifty to sixty miles to the west, at Manatí, similar weather at this time and similar abundance of egg-clusters and parasitism paralleled those of the Loíza, Canóvanas and Río Grande region. Between Loíza and Manatí, in the Plata River valley, at Toa Baja and Dorado, the same weather had the same preliminary effect, but in the following weeks a tremendous increase in total number of eggs and parasitism developed, far greater than any other time until 1941. Some connection should exist between this heaviest rainfall in any one week at Dorado in the five year period, and the greatest number of egg-clusters and the highest parasitism recorded, yet the lack of any comparable result at the localities on either side, one having four inches more and the other four inches less of rainfall, would appear to indicate that it was nothing but a coincidence. Or, if not a coincidence, at least a result that has not been duplicated or paralleled at any time during the investigation at any other locality in any other part of the Island.

On the contrary, at other localities, one finds peaks of egg-clusters and parasitism developing just before heavy rains, in which case the supposed result comes before the cause. Or the exceptional rains have no apparent effect. After the exceptionally dry year of 1939 on the south coast, 1940 proved to be exceptionally wet, and at Ponce and at Santa Isabel, no peaks of egg-clusters appeared during the entire year of 1940. At Guánica, the highest peak of the five years came in the late winter of 1940, of which a very modest parasitism by *Trichogramma* was supplemented by an all-time high of 20 egg-clusters per man-hour "eaten by ants".

All of the cases cited are extremes of rainfall, or abundance of host egg-clusters, or parasitism, or "eaten by ants", and if these make no consistent pattern of cause and effect, it seems hopeless to expect minor variations of weather to produce an effect. Furthermore, if one can not be sure of the results of rainfall after all the records are in from all over the Island on a five year basis, it is apparent that any attempt to predict results in advance, based on rainfall, has no more than even chances of being correct.

#### TEMPERATURE

Aside from specific factors in an environment, those of most general

importance to an organism are humidity and temperature. With rainfall, at least within the limits normally experienced in Puerto Rico, so modified by irrigation that humidity is apparently eliminated as decisive in affecting *Diatraea* oviposition and parasitization by *Trichogramma*, the factor of temperature remains to be considered. In temperate zone countries, temperature is often the deciding factor for all of the activities of an organism, but in the tropics, generally rainfall is of major importance to living things. When, as in the present investigation, irrigation upsets all this, the apparently minor factor of temperature must at least be evaluated. The generally lesser importance of the temperature factor is indicated by the absence of Weather Bureau records at some vital points where we made observations. When the effect of temperature on *Diatraea* oviposition began to be considered, recording thermographs were obtained, and thru the courtesy of the Centrals concerned, were operated for the remainder of the period at Hda. Santa Rita, Guánica, at Hda. Florida, Santa Isabel, and at Hda. Verdaguer and Hda. Olimpo, Guayama.

One can hardly think of a positive stimulus having a negative effect, but it is not so difficult to imagine a lack of stimulus having no effect. Fajardo has the least recorded variation between day and night temperatures,—by months, less than 20°F.— for the 1936-41 period, and also has the smallest number of *Diatraea* egg-clusters per man-hour, the least variation in their abundance, and the lowest parasitism by *Trichogramma*. If all of these negatives mean anything, then it seems possible that their opposites might be correlated. Thus we awaited results of the thermograph installed at Hda. Florida, Santa Isabel, for the fields in that region have the greatest variation in abundant of *Diatraea* egg-masses, the greatest abundance of eggs and the highest parasitism by *Trichogramma*. For the period during which the thermograph was operated, the variation between night and day was almost exactly the same as for the same period at Fajardo. Obviously, the apparent correlation at Fajardo was meaningless as applied to Santa Isabel, and the extremes of *Diatraea* oviposition at Santa Isabel are not to be explained on a temperature basis.

Temperatures at Santa Isabel do not differ decisively, and often not at all, from those reported at Ponce and Aguirre, and certainly can not be considered as explaining the higher oviposition of *Diatraea* moths there as compared with adjacent localities to the east and the west.

At Santa Rita, Guánica, no two years of our observations exhibit a similarity of pattern of abundance of egg-clusters or attack by parasites

or predators, and the records of temperature were obtained for too short a period to indicate anything.

During the third year of observations in the Guayama region, it was noted that an abundance of egg-clusters and accompanying high parasitism by *Trichogramma* developed first in the fields farthest away from the coast and closest up under the mountains, and, by going over the records of the previous years, it was possible to trace a similar occurrence during those years. It seemed possible that this might be an effect of temperature, thus two thermographs were installed in addition to the maximum-minimum thermometer records contributed by the office of the Irrigation Service at Guayama: one at Hda. Verdaguer, not far from the coast of the Caribbean, the other at Hda. Olimpo, up the Carite valley between the mountains. Most unfortunately, little difference in the time of appearance of abundance of egg-clusters was noted in the two final years for which comparative temperature records are available. The thermograph records show but minor variations from each other, or from the maximum-minimum thermometer readings in town.

If one finds an apparent correlation between *Diatraea* egg abundance and temperature at one locality, it can often be almost exactly duplicated in reverse at the same locality another year. Thus, the net result of all observations on temperature, including the special observations in addition to those furnished by the Weather Bureau, fails to indicate anything decisive. Indeed, one may state that: within the normal range of temperatures and humidities experienced in the coastal cane-growing regions of Puerto Rico, oviposition by *Diatraea saccharalis* F., and parasitization of its eggs by *Trichogramma minutum* Riley most of the time, depends on other factors than rainfall and temperature.

#### LOCALITY GRAPHS

Shortly after the investigation reached the stage of establishing the boundaries of the regions that were to be observed, and restricting observations only to those localities, the construction of graphs for each locality was begun, to show graphically the conditions as they were observed. Begun in a small way, with horizontal spaces equivalent to weeks, as the investigation was continued from year to year, additional paper had to be pasted to the original sheets to continue to represent the accumulating data. Vertical spaces were egg-clusters per man-hour; the fresh and the hatched on one side of a central base line; the eaten by ants and the parasitized and the emerged (including partly parasitized) on the





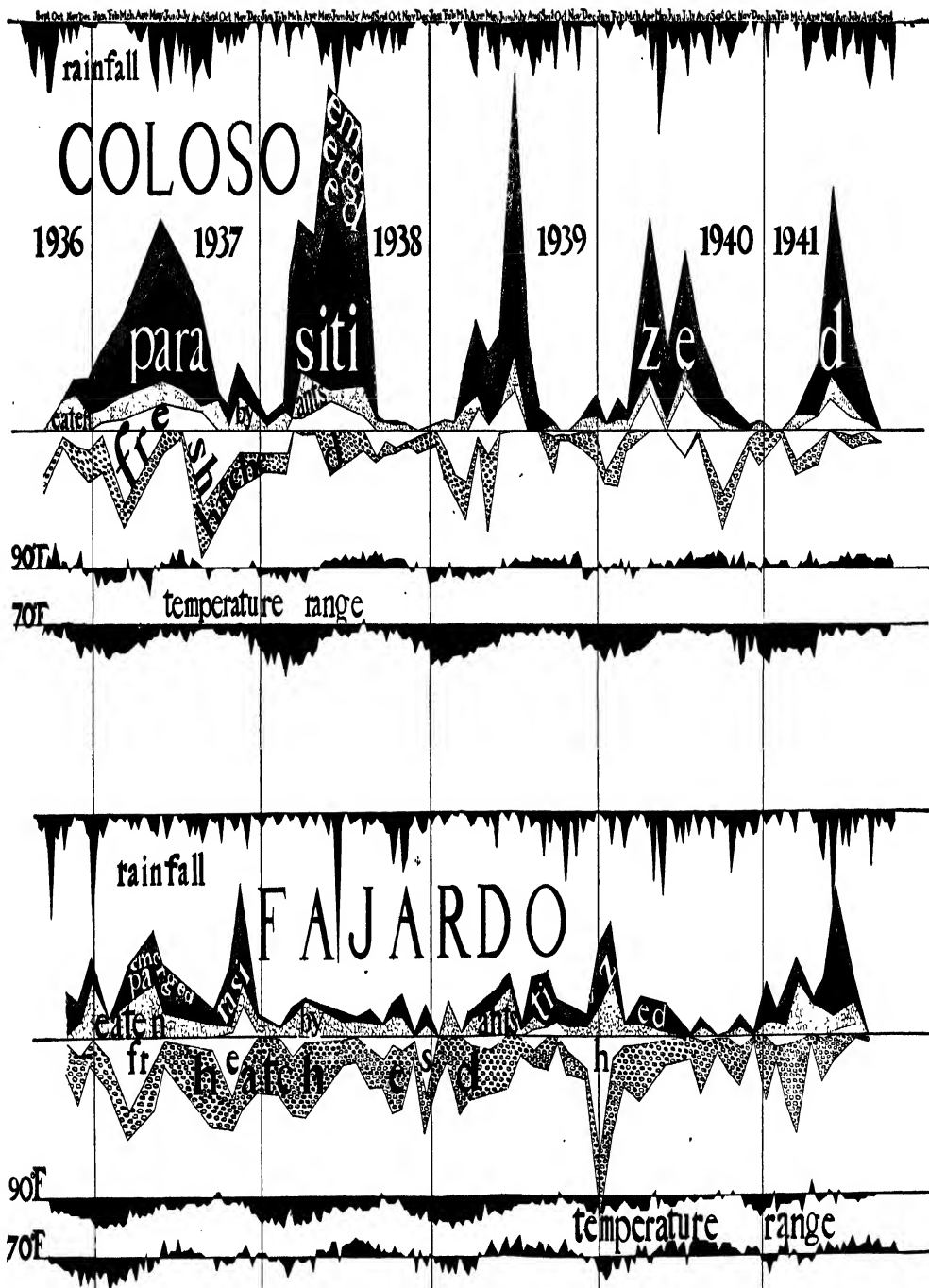


Plate 1. Comparative abundance of the egg-masses of *Diatraea saccharalis* F. in the northwestern corner (Coloso) of the Island of Puerto Rico, and in the northeastern corner (Fajardo).

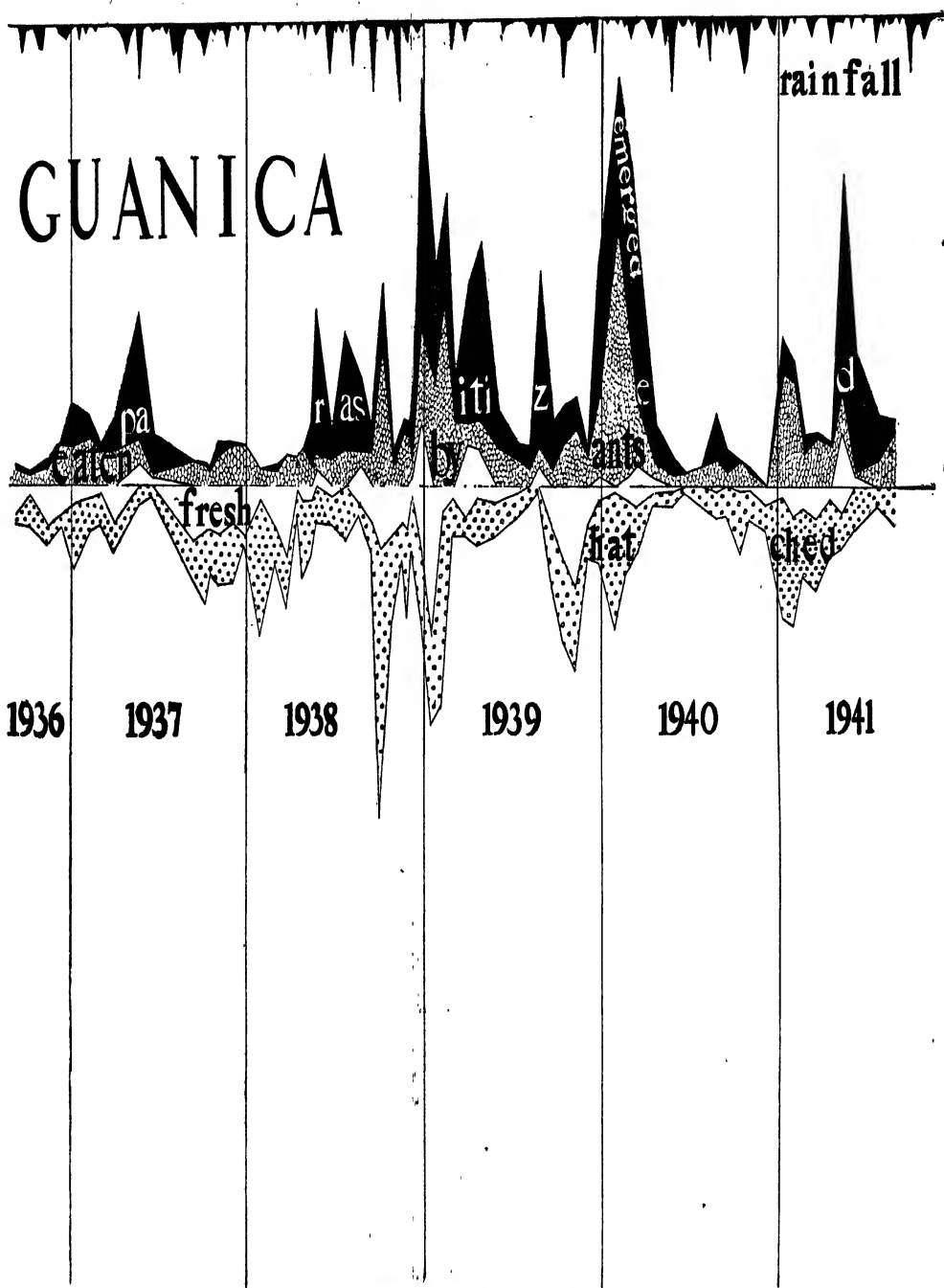


Plate 2. Abundance of egg-masses in the southwestern corner (Guanica) of Puerto Rico.

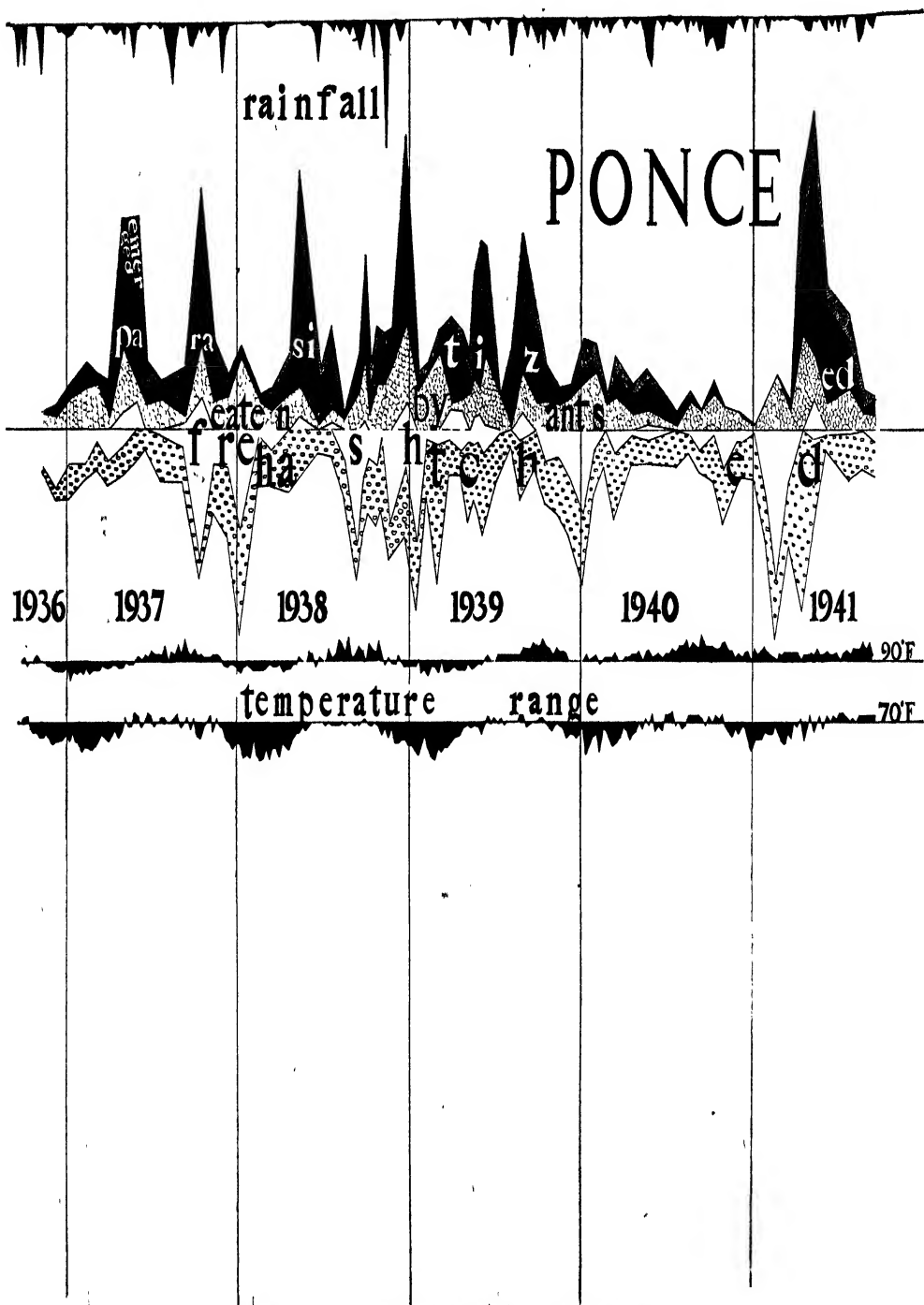


Plate 3. Abundance of egg-masses at Ponce, on the south coast of Puerto Rico.

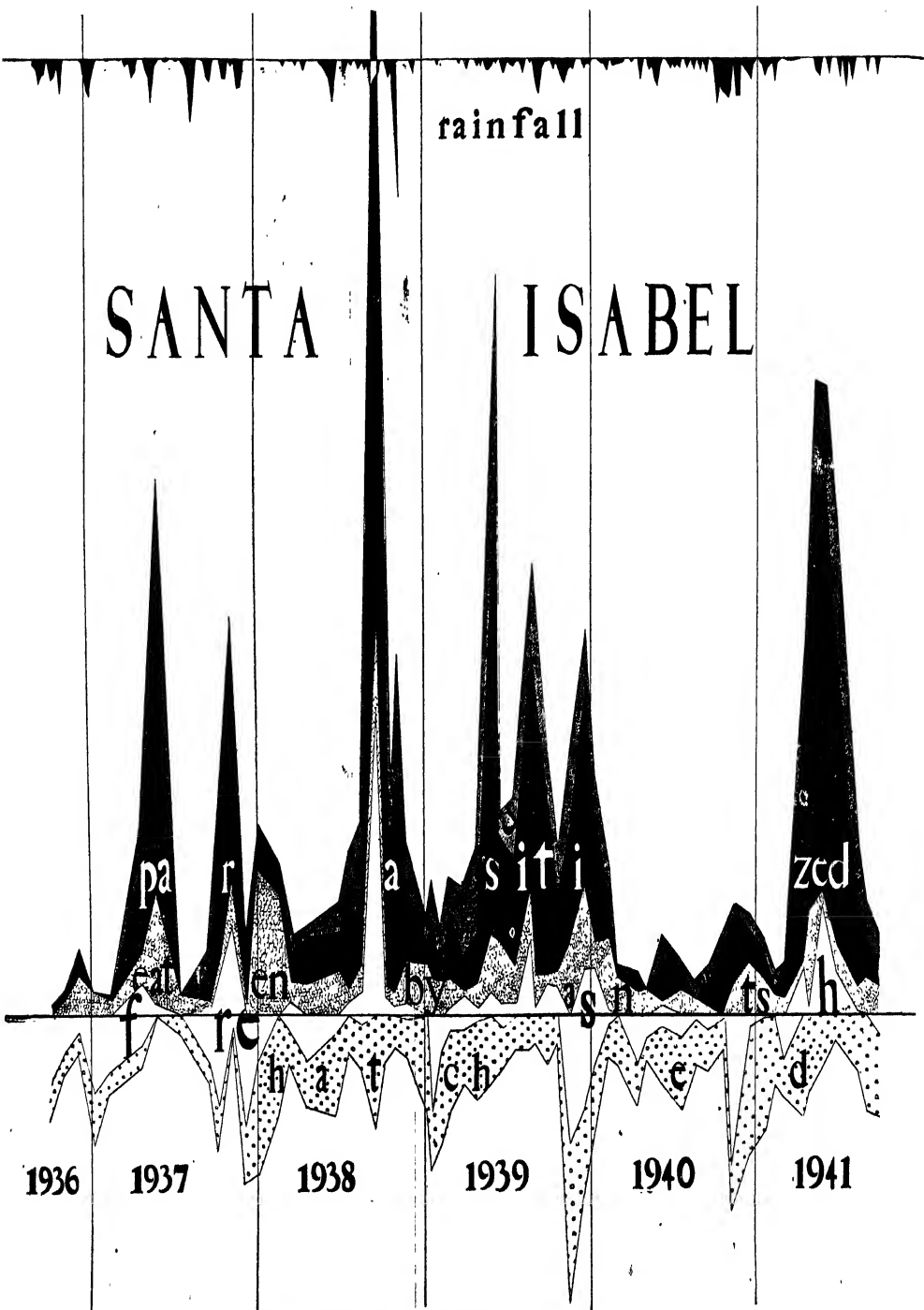


Plate 4. Abundance of egg-masses at Santa Isabel (adjacent to Ponce) on the south coast of Puerto Rico.

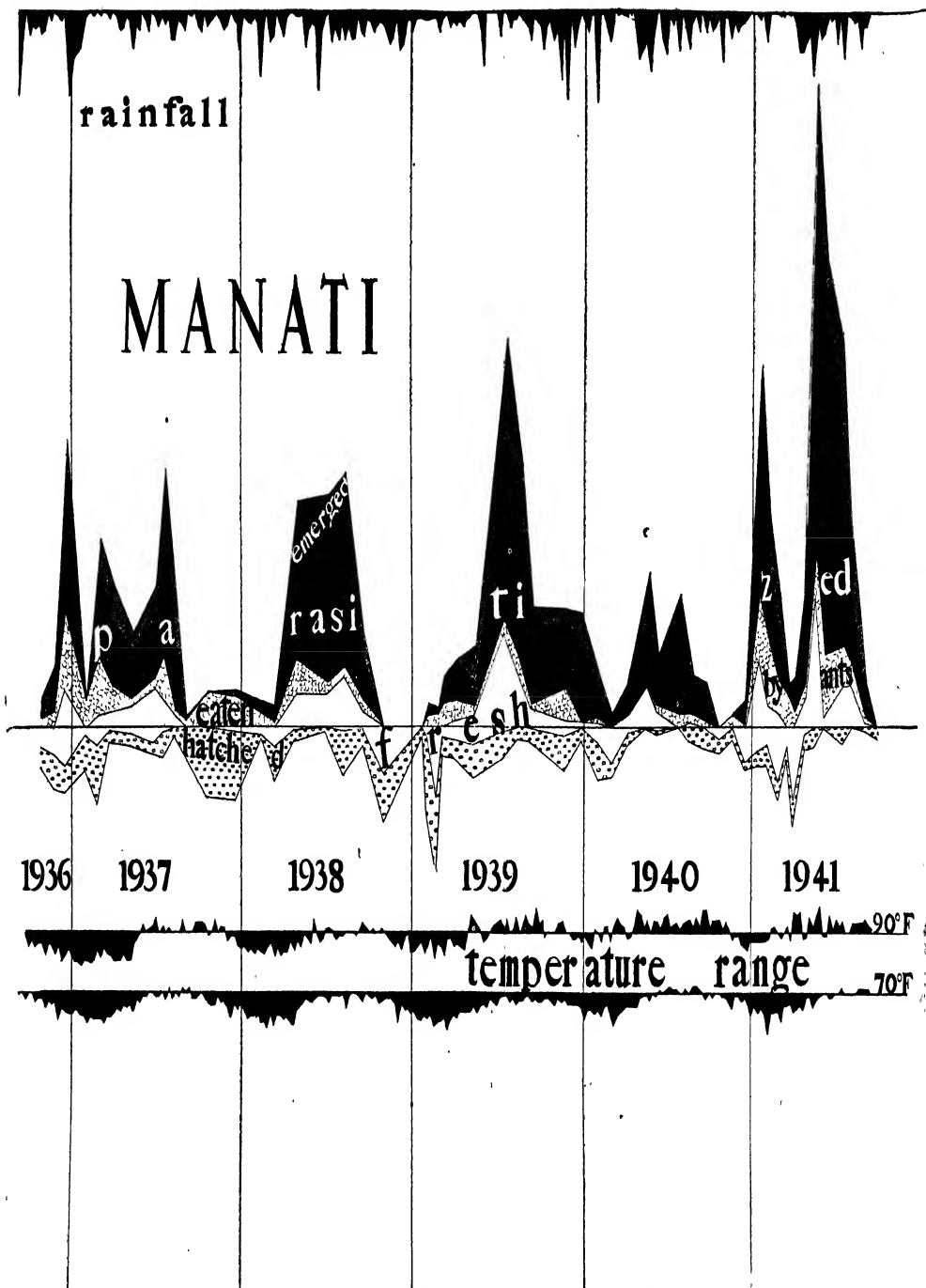


Plate 5. Abundance of egg-masses at Manati, on the north coast of Puerto Rico

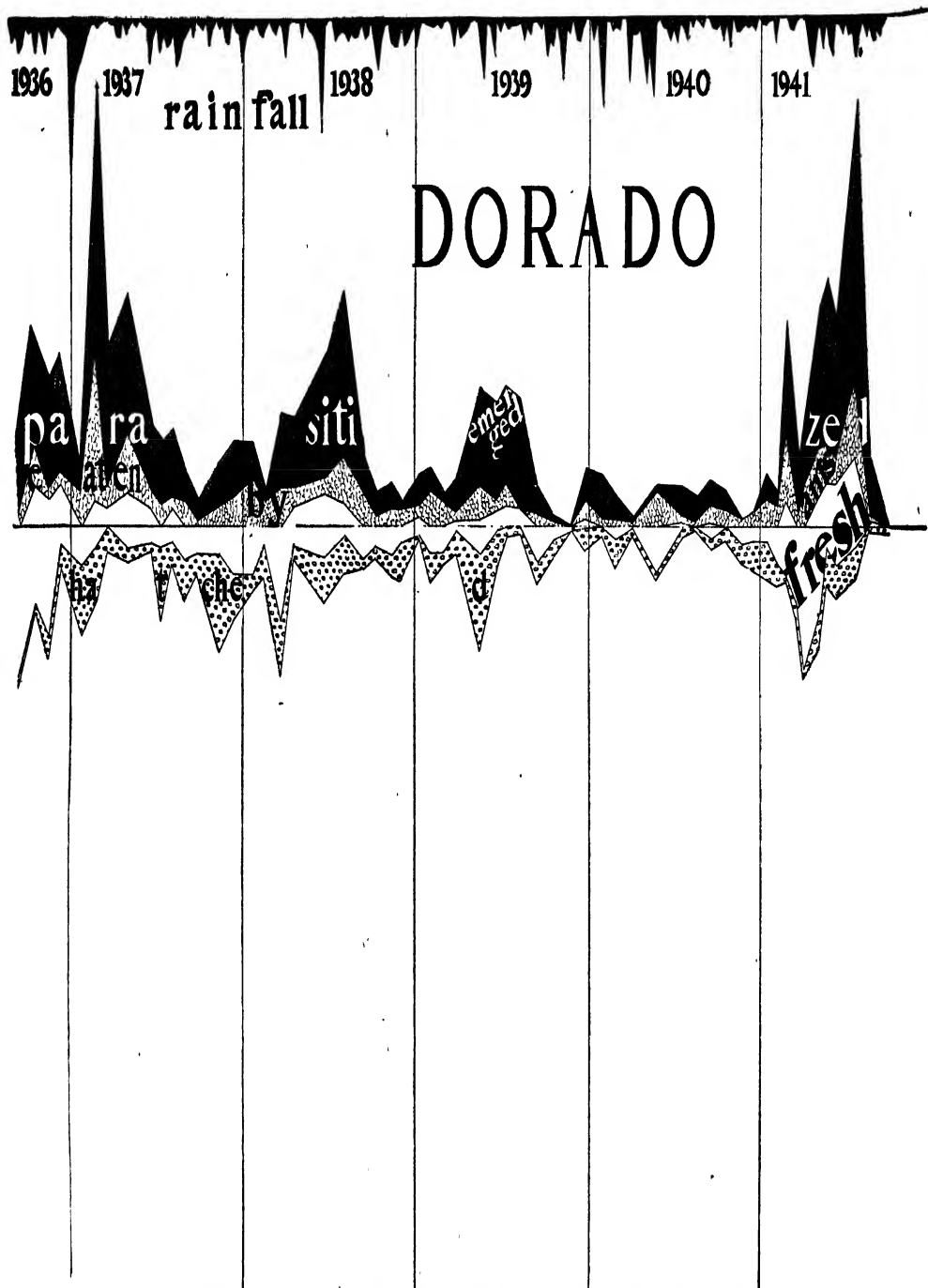


Plate 6. Abundance of egg-masses at Dorado (adjacent to Manatí) on the north coast of Puerto Rico.



other side representing natural control. From the very beginning, rainfall by weeks was entered on what it was expected would continue to be blank space, and later the maximum and minimum temperatures by weeks. In this way, all pertinent data for each locality was kept graphically up to date by being entered on the graph just as soon as the field notes had been summarized and the weekly weather reports received.

Some of the more interesting of these graphs have been redrawn, and are here presented. In all these redrawn graphs, the central base line divides "fresh" egg-clusters into those above the line which were actually parasitized or would have become parasitized had they been left in the field, and those below the line which would have hatched had they been left in the field. Thus, everything above the central line represents egg-clusters subject to natural control; everything below the line represents surviving *Diatraea* caterpillars.

The first plate of graphs includes that for Coloso, typical of the northwestern corner of Puerto Rico, which accompanies that of Fajardo, of the northeastern corner, and that of Guánica of the southwestern corner: no two of which show any traces of similarity. The graphs of two north coast regions, Manatí and Dorado, show the general similarity of two adjacent regions and minor differences; the graphs of two similarly adjacent south coast localities, Ponce and Santa Isabel, are presented together for contrast.

#### IDEAL GRAPHS

The ideal curve of host abundance and control by parasitism would show these characteristics: starting from a scarcity of host, it should show a rapid rise in host abundance, followed by a parallel rise in parasitism which continues at almost total parasitism until fresh hosts almost completely disappear, which of course involves the coincident disappearance of the parasites. Applied to the graphs of *Diatraea* egg-abundance, the fit is almost perfect in 1938 at Coloso and intervening localities to Manatí and Dorado, and only a little short of perfect during the other years from Coloso to Arecibo. (It also describes with equal accuracy the major wave at Guánica in the winter and spring of 1940, but with ants playing the important role in control and *Trichogramma* of secondary importance). The recurrence of this wave each year at approximately the same time in northwestern Puerto Rico would appear to indicate that it is a seasonal phenomenon in its initiation at least, being accompanied by a more or less pronounced difference in rainfall and temperature in the spring. The



more or less sudden drop before the end of summer, long before there is any difference in temperature or rainfall, would therefore appear to be due to the perfection of natural control, and not to a change in weather, for there is none at this time of year. The height of the wave, its continuance and its sudden break are all aspects of the near perfection of control by parasites, and it is the combination of seasonal initiation and natural control which forms an adequate explanation of the waves of abundance of *Diatraea saccharalis* in the northwestern corner of Puerto Rico.

#### ACCURACY

The difficulty in explaining the locality graphs for the remaining four-fifths of Puerto Rico on any reasonable basis raises the question whether these graphs actually are an accurate representation of conditions in the cane fields. Of course, the same methods were used in the Coloso-Arecibo region as elsewhere, but there remains the possibility that methods sufficiently accurate there, fail to give adequate results elsewhere. It must be admitted that observations every month did not come often enough to correctly plot waves of short duration, and also the erratic character of the waves of abundance might be due in part at least to a change in the actual fields from month to month, as some became too high for ready examination and were replaced by others of much younger cane. It must be remembered, however, that "height of cane", as such, does not affect the result at all, for the summarized data of height of cane is statistically conclusive that there is no effect. Individual fields vary, and sometimes the variation is so extreme that there seems to be no similarity. Some fields may be in the trough of a wave when others are at the crest, the average of such fields producing such confusion as to obscure the pattern of each one. This will possibly explain how it is possible for waves of abundance of egg-clusters to appear in mid-winter at Manatí, just as one is sure that perfection of natural control should make any such thing impossible. It is impossible in the fields where it has been attained, but other fields now being examined completely change the picture, especially if the region is not a natural group.

#### A SINGLE FIELD

It has been taken for granted that once *Trichogramma* becomes established in a field, it will continue to increase indefinitely until all eggs are parasitized. In Puerto Rico, this may not occur.

As an outstanding single example, one might cite the case of Santa

Rita No. 4 at Guánica, which as plant cane a month and a half old on December 7th, 1938 was observed with no egg-clusters, but twenty-two days later had 160 egg-clusters per man-hour and 51% parasitism. One should note that this happened in December, at a time too late for cutting seed cane for planting gran cultura, and too early for the regular grinding season, if it is thought that the moths flew in from some field of mature cane, disturbed when the cane was cut. By the ideal curve, parasitism should increase, and the number of egg-clusters decrease only when close to 100% parasitism had produced natural control. Instead, when examined three weeks later, both the number of egg-clusters and the percentage of parasitism was half what it had been before, with the situation practically unchanged when again examined at the end of another three week interval. Two weeks later, the number of egg-clusters was down to one-sixth of the peak, but of this decreased number parasitism had gone up to 40%. The peak of 160 egg-clusters per man-hour is the all time high for plant cane in Puerto Rico, and the field is an exception to that extent, but it is typical of many in that an unprecedented high of fresh egg-clusters to be parasitized actually was not attacked, for *Trichogramma* was absolutely and relatively much less abundant when the next two examinations were made, and absolutely least abundant at the final examination.

This field is like a poorly-planned drama, with all the interesting action happening off-stage between the first and second act. But with no egg-clusters collected at the first examination, how could anyone imagine that the next observation would show an all-time high? In other years, this field had shown no exceptional abundance of egg-clusters, and when last observed on June 3d, 1941, only four egg-clusters were found in an hour's search, while on June 16, 1937, only three egg-clusters had been found in an hour. Thus, we may be sure that a field which once has an abundance of egg-clusters doesn't always have an abundance, and apparently is as unpredictable as where lightning will strike. Yet the third examination in 1938-9 gives the second all-time high at Guánica for plant cane, and the fourth examination the third all-time high at Guánica. The decrease from the previous highs still o'ertopped all its neighbors for five years! Thus, altho there was a relative decrease in the number of eggs, this was an abundance subject to parasitism many more than in any other field in the region at the time. (In December 1938, the black egg-parasite, *Prophanurus alecto* Crawford, had just made its only recorded appearance at Guánica, and it had entirely disappeared in January, when *Trichogramma* was merely less abundant).

## UNPREDICTABILITY

If the locality graphs of northwestern Puerto Rico actually represent commercial control of *Diatraea* by its egg-parasite until the following spring, this requires four or five months of continued abundance of host eggs, as well as of the parasite, before it becomes effective. This is shown by the sudden drop in abundance of host eggs, approaching zero, and continued scarcity thru the remainder of autumn and winter until the following spring. By contrast, the sharp-pointed and narrow-based waves of *Diatraea* and *Trichogramma* abundance almost invariably occurring on the south and east coasts are decidedly not of the same character. To be sure *Trichogramma* has often parasitized most of the eggs, but if it takes four or five months to produce really effective control at Coloso, the same result can not be obtained elsewhere in much less time. Thus we may conclude that the sudden decrease in abundance of host eggs is not due to parasitism of host eggs of the preceeding generation, but would occur in any case, and is largely or entirely independent of parasitism. In fact, it also occurs where parasites are scarce, in which case, ants tend to fill the vacuum, as well as when parasites are just beginning to become abundant, or are numerous. That is: waves of abundance of *Diatraea* eggs occur in the greater part of the canegrowing area of Puerto Rico for no apparent reason at any particular time, and tend to disappear with equal suddenness without apparent reason. Furthermore, that the larger part of such waves is destroyed by parasitism or eaten by ants is merely an accident that gives no indication of how soon that particular wave will recede, or how long the succeeding trough of scarcity of eggs will continue.

## STIMULI TO OVISPOSITION

In captivity, the female moths of *Diatraea saccharalis* live only a few days, but during their short adult life they deposit large numbers of eggs on everything in their environment, showing little discrimination between cane leaves, grass, strips of paper, or the glass sides of the tube. As to what happens in the field, we can only guess, for our search for eggs was conducted only in cane fields, and only exceptionally and by accident did we sometimes happen to find an egg-cluster on a blade of malojillo grass. In Puerto Rico at least, if the eggs are undisturbed by ants or wasps, they almost invariably hatch, as practically no dried-up or infertile eggs were noted.

Nothing is known as to the stimuli affecting oviposition in the field. To the extent that eggs are usually laid in rows, one has the feeling that some care was used in oviposition; but sometimes they are laid without any order in an irregular mass that suggests that no external stimulus to oviposition was needed, as the moth was in so much of a hurry due to internal pressure that there was no time to even lay them straight. In captivity, moths lay from 30 to 40 masses of eggs, and quite possibly do even better than that in the field. Rosenfeld & Barber (1914) note one female in Argentina that laid 72 masses in five days, but some of these masses contained very few eggs. On this basis, it is obvious that not many moths are required to result in a "wave of oviposition," really quite an insignificant number by comparison with that of most other insect pests attacking crops. Indeed, the moths are apparently so scarce in the field, or so difficult to find, that the number of egg-clusters per man-hour gives a much better idea of the abundance of the insect. But it fails to answer the question of whether an abundance of eggs is the result of a stimulus acting on a comparatively few moths, or if it was a stimulus acting on pupae that many moths should emerge at about the same time, or how far back the stimulus must be carried, and acting on what stage of the insect, to produce what we find as an abundance of egg-clusters. Where climatic conditions are more extreme than in coastal Puerto Rico, the effects on *Diatraea* promptly become apparent in all stages of its existence, but under the equable climatic conditions of the cane-growing regions of Puerto Rico, a major response, as expressed in an abundance of moth-borer egg-clusters, is apparently the result of a temporary and partial failure of biologic control in a previous generation. Nowhere in Puerto Rico at any time since entomologists have been here has *Diatraea* even begun to completely occupy its niche and completely destroy a crop of cane. Before the introduction of the Surinam toad, *Bufo marinus* L., such complete occupancy by another insect pest of cane often did occur in Puerto Rico when white grubs ate all cane roots. Only in some coastal valleys of northern Perú (Wolcott 1929) has *Diatraea* been observed to cause 100% damage to mature cane, and this despite the presence there of fully as many parasites as are present elsewhere. Natural control by parasites and predators is so effective in northwestern Puerto Rico that *Diatraea* practically disappears by midsummer, and elsewhere is merely less perfect, and is devoid of any seasonal pattern. In either case, *Diatraea* fails to occupy more than a small fraction of its distinctive niche in Puerto Rico cane fields.

## PHASE OF MOON

On the possibility that phase of the moon might have a decisive effect on oviposition by *Diatraea* moths, all of our records were summarized on that basis. The vital difficulty proved to be to allocate the eggs according to the phase of the moon in which they were laid, for the records gave no indication of whether a "fresh" egg-mass was laid one or seven days before, and one could be even less exact regarding the age of any other kind of egg-cluster. With an arbitrary approximation as to the supposed age of the egg-clusters, the results were positive in showing most eggs laid when the moon was "new" (dark), and least when it was full, but with so little difference in the totals that the results were by no means conclusive. Waves of egg-clusters do not come at monthly intervals, or, at least as we made observations at five, four and three week intervals, any such monthly waves were obscured by larger waves at somewhat or very much greater intervals.

## VARIATION

The sharp points of the crests of waves in a locality graph represent the average of several fields, the field with most egg-clusters often having twice or many times as many as the field with fewest. Usually the difference between them was considerable. All the details of the graph are also an average, in some classes glossing over differences so great that one might better call the result a compromise. To give some idea of how great are these extremes, those over the entire period of five years are given in the accompanying table (Table No. 2, p. 64): the maxima for both plant and ratoon, the average of the ten highest, the minima for plant and ratoon, the latter indicating how many zeros were found at each locality. It must be admitted that the figures for each locality are not entirely comparable, for the number of fields examined varied from 440 at Guayama-Arroyo and 422 at Ponce, to 215 at Coloso and 213 at Isabela and Quēbradillas, but any correction would only increase the proportion of zeros where they are already most numerous as shown in the table. No correction is possible for the maxima, for even one additional record at a locality might upset its best previous record.

The figures of the average of egg-clusters in the fields with the ten highest records for plant and ratoon at each locality give a better idea of how many high fields may be expected after averaging down the ex-

ceptionally high peaks, but are often strikingly persistent in paralleling the extremes, as note Ponce and Guayanilla, Fajardo and Dorado.

Manatí plant fields in 28 instances had no egg-clusters, and Fajardo ratoon fields in 22 instances had none. Of the field of plant cane at Guánica with 160 egg-clusters per man-hour, a detailed discussion has already been given, stressing its inconsistencies. The field at Maunabo (in the Yabucoa-Maunabo-Patillas region) previously called Garona or Bordalesa at Km. 110.5, on November 31, 1938, had the all-time high —152 egg-clusters— for ratoon cane for the entire Island, just following the second (100 egg-clusters), and followed immediately by the third (78) and fourth (64) for its region as well. These records coincided with the only observed appearance of *Prophanurus* in this part of the Island. This does not necessarily mean that it always has an abundance of egg-clusters, for in August 1939 only 8 per man-hour were collected, and less than a month before its second high, it had but 18. It is entirely consistent in parasitism, however, with as low as 25% for 8 egg-clusters, with perfectly graduated increases to the highs at 80%. Even with maximum parasitism, 6 egg-clusters that had hatched were found, thus it failed of 100% actual control by *Trichogramma*. But it is so largely consistent as to serve as an even greater contrast to the inconsistencies of Santa Rita. No. 4, and many similar fields at Guánica and elsewhere on the south coast.

Both of these highs, in plant cane and in ratoon, came at a time when egg-clusters were generally abundant nearly everywhere, but in the Guánica district on the same day one field had only 10 egg-clusters per man-hour, or only one-sixteenth of the number found at Santa Rita No. 4, and at Patillas on the same day, one field had 29 egg-clusters, or one-eighth of the number at Garona.

#### UNPREDICTABILITY IN PARASITISM

It has been previously shown how the average of parasitism increases regularly with increases in the number of egg-clusters. This is only by averages, however, and the variations in total number of egg-clusters, as noted above, are merely symptomatic of an equal variability in parasitism in both directions. While the majority must be sufficiently consistent if the averages are to follow a regular pattern, many individual fields do not follow this pattern. Not only are fields with few egg-clusters and high or total parasitism to be found, but also some with large numbers of egg-clusters and little or no parasitism. This is the realistic, practical background for the entire investigation, for in such fields laboratory-reared

parasites can be released with reasonably good chances that they will promptly and decisively increase parasitism. Indeed, it is because of the great variation in conditions in individual fields in Puerto Rico, not following a set seasonal pattern except in the northwestern corner where natural control is most nearly effective, that success in the economic aspects of this project is possible.

Our experiments have indicated, as indeed common sense would suggest, that release of laboratory-reared parasites is likely to result only in failure when fresh host eggs are scarce, or, if fresh eggs are abundant, can not be proved to be due to the release when natural parasitism is high. From the practical, commercial standpoint, attempts at the biological control of a pest when it is so scarce are hardly justified, while fields with high natural parasitism certainly do not need additional parasites. Put on a statistical basis, success is hardly to be expected if less than five fresh egg-clusters can be found in an hour's search, and can not be proved if natural parasitism is over 33%. These are the absolute minimum conditions, with results more decisive if parasitism is very low or non-existent, and more likely to be successful if the abundance of egg-clusters considerably exceeds the minimum of five fresh ones.

#### FIELDS FOR RELEASES

Of fields meeting these minimum conditions, 445 were noted during the five years observations in Puerto Rico, out of a total of 4,707, approximately every tenth or eleventh field. Evenly distributed, finding such conditions might seem difficult, and indeed the real purpose of the latter part of the investigation was to find some sign or indication of how they might be predicted without continued inspection of all fields. We found no such sign or indication, and their occurrence can not be predicted except in a general way, but a preliminary inspection of ten minutes is sufficient to indicate all that one needs to know of a particular field, in practise. If no fresh egg-cluster is found in the first ten minutes of inspection, there is little point in looking further. If one fresh egg-cluster is found in the first ten minutes, conditions at least come up to the irreducible minimum; if two or more fresh egg-clusters are found, the field is worth more careful inspection, and in most cases will be found suitable for releases. Actually, their distribution is by no means uniform as to locality or season. In 100 fields of plant cane, 12.4 are of this character, but of ratoon fields only 6.4. Only at Dorado and Toa Baja, of all the regions examined, was the number of such fields equal in plant

and ratoon: 11 in 100 fields. Twice as many fields of plant cane at Ponce, Salinas and Guayama were suitable for the release of parasites as of ratoon cane, and Santa Isabel had four and a half times as many. Even greater were the contrasts in the northwestern corner of the Island, and Coloso had no fields of ratoon cane of this character, except one where the trash had been burned.

During the month of July, hardly 1 field in 100 meets these conditions, only 2 in 100 in June, and hardly 3 in 100 in August. April and May have more of these exceptional fields, but it is only beginning in September and October that they constitute a tenth of all fields; in February and March average 12 in 100; 13 in November and January, and reach a high of 14 in 100 in December. By localities, only 4 such fields were found in 100 examined at Coloso, where the near perfection of natural control makes their occurrence least likely, and 5 at Arecibo and Fajardo, but Ponce and Guánica had 11 in 100, and Salinas 17. Combining locality and month, January at Ponce had 39 such fields out of 100 examined in 1936-41, Santa Isabel 37 in November, Salinas 34 in February and Guánica 30 in November. These figures only show what was observed in 1936-41, and give only an indication of what may be expected in other years, but in general are so consistent as to indicate a strong probability that during any of the autumn, winter and early spring months of any year one should find one out of every four or five fields suitable for the release of parasites at these localities. To this limited extent in Puerto Rico therefore, temperature, or season, does have an effect on *Trichogramma* even if not on its host. The chances are only slightly less of finding such suitable conditions at most localities on the east and northern coasts during the winter, and while plant cane is most likely to offer suitable conditions for release, ratoon cane needs them with sufficient frequency not to be entirely neglected.

Experimental releases have an equal chance for success at any locality, regardless of how low are the chances of finding suitable conditions at that locality at that time, if a field can be found which meets the requirements of an abundance of fresh egg-clusters and low parasitism at the time of release. It certainly is like looking for a needle in a haystack, however, to attempt to find the exceptional field in mid-summer, and in practise, attempts at release should be confined to the autumn, winter and spring. The northeast coast, because of scarcity of egg-clusters at all times of year, and the northwest coast, because of normal near perfection of natural control, offer the least opportunity for conducting successful



releases. The south coast during the fall and winter has the greatest number of such exceptional fields with many fresh egg-clusters and low parasitism, and offers the best opportunity for the successful release of laboratory-reared parasites in Puerto Rico.

#### SUMMARY

To determine the optimum conditions for releasing laboratory-reared egg-parasites, *Trichogramma minutum* Riley, in Puerto Rican fields of sugar-cane for the control of the lesser moth borer, *Diatraea saccharalis* F., field observations on natural conditions of parasitism at sixteen coastal localities were commenced in the autumn of 1936, and continued until the autumn of 1941.

For a locality record, observations of one hour or one-half hour were made at four or five week intervals, later at three week intervals, in from five to eight or more typical fields, not adjacent, but not too far distant from the point where rainfall and temperature records were made by co-operative observers of the Weather Bureau.

Of the 45,430 egg-clusters collected from 4,595 fields, 5,391 had had parasites emerge from them, 638 were partly parasitized and 11,433 were parasitized: a total of 17,462 for parasitism, or 38% of the total. At all localities, and during all months, *average* parasitism increased with abundance of egg-clusters.

Egg-clusters eaten by ants, *Monomorium carbonarium ebeninum* Forel, totaled 7,377, or 17% of the total.

Egg-clusters from which caterpillars had hatched numbered 12,635, or 28% of the total. Unhatched, apparently unparasitized or "fresh" egg-masses numbered 7,965, or 18% of the total. The presumed fate of these "fresh" egg-clusters was not in proportion to the collected number of hatched, eaten and parasitized, because, as pointed out by Dr. G. W. Kenrick, so many parasitized and eaten eggs had already been subtracted. Had they not been collected, but left in the field, he estimated that somewhat more than half would hatch, nearly one-sixth be eaten by ants, and nearly one-third become parasitized. Adding these to the observed number in each class indicates that approximately 38% of all *Diatraea* eggs hatch, 21% are eaten by ants and 46% become parasitized, or with some eggs in both classifications, a total for natural control by ants and wasps of 62%.

Young plant and ratoon cane, from 18 inches to four or five feet in height only, was examined. Height of cane within these limits had

no effect on abundance of egg-clusters or parasitism. The percentage of parasitism in plant cane was 27%; in ratoon cane of which the trash had been burned 31%. In ratoon cane of which the trash had not been burned, natural parasitism was 50%.

Nearly half of the environment of the fields examined was high cane, and sugar-cane in some stage of growth formed two-thirds of the environment.

Within the normal range of temperatures and humidities (rainfall plus irrigation) experienced in the coastal cane-growing regions of Puerto Rico, *Diatraea* oviposition, and parasitization by *Trichogramma* most of the time, depends on other factors than rainfall and temperature.

The initiation of the typically broad-based, blunt-topped waves of abundance of *Diatraea* eggs and high parasitism by *Trichogramma* observed each year in the northwestern corner of Puerto Rico, is presumably seasonal, being due to the coming of spring, but their height, duration and sudden break before autumn are all aspects of the near perfection of natural control by parasitism in this region.

In the remaining four-fifths of the coastal cane-growing area of Puerto Rico, sharp-pointed and narrow-based waves of abundance of *Diatraea* eggs occur for no apparent reason at any particular time, and disappear with equal suddenness without apparent reason. That the larger part of the eggs in such waves is often destroyed by parasites or eaten by ants is merely an accident that does not imply adequacy of natural control in the egg stage at that time to account for their disappearance.

Maxima of 160 egg-clusters per man-hour in plant cane and 172 in ratoon cane, and many instances of minima of zero are symptomatic not only of variation in abundance of egg-clusters, but also of deviation from the average of parasitism in proportion to the abundance of host eggs. Conditions of abundance of moth borer egg-clusters in any particular field can be determined only by inspection, and no specific prediction can be made based on vigor or age of cane, weather, location of the field, environment, or any other kind of sign or indication that we have been able to detect. Fields with more than five fresh egg-clusters per man-hour of examination, and parasitism of not more than 33% constituted nearly a tenth of all fields. Such fields offer the best opportunity for the effective release of laboratory-reared parasites, and are the only ones in which releases can be commercially justified.

Generalized predictions as to the occurrence of such conditions can

be made. They occur twice as often in plant as in ratoon cane, and are scarce almost to the vanishing point in mid-summer. They are most abundant on the south coast during autumn, winter and spring, with a peak of abundance in December, and it is here and at this time that releases made in fields selected on the basis of previous inspections will be most successful.

#### CONCLUSION

The initiation in the formation of waves of abundance of egg-clusters of *Diatraea saccharalis* F. each spring in the northwestern corner of Puerto Rico is seasonal, but their height, duration and sudden drop in late summer are due to the near perfection of natural control by *Trichogramma minutum* Riley. In the remaining four-fifths of Puerto Rico, the factors responsible for the initiation, height and usually much shorter duration of waves of abundance of egg-clusters are not seasonal at all, but apparently depend on temporary and partial failure of biologic control in previous generations of the host. Natural control in the egg stage, even with often almost as many egg-clusters eaten by ants as attacked by parasites, rarely occurs, because of the shortness of the period of the wave. Irrigation so modifies humidity that rainfall, varying from less than 30 inches to nearly 90 inches per year, can not be proved to be a factor, and the variations in temperature are within too narrow limits to produce an effect. Height of cane and variety of cane has no effect on egg-clusters, but ratoon cane averages greater abundance of host eggs, and higher parasitism, than plant cane or ratoon cane of which the trash has been burned. Parasitism invariably averages higher when or where host eggs are most numerous, but great variation in abundance of eggs in individual fields is paralleled by comparable variation in parasitism not depending on host abundance. Fields with more than five fresh egg-clusters per man-hour and less than 33% parasitism are one out of every ten or eleven, and generalized predictions as to the occurrence of such conditions can be made. The release of laboratory-reared parasites can be commercially justified only in fields meeting these conditions, which occur most often in plant cane on the south coast during the winter.

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# THE SEASONAL CYCLE OF INSECT ABUNDANCE IN PUERTO RICAN CANE FIELDS

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Even the most single-minded entomologists find difficulty in so exclusively concentrating on a single insect as to avoid noticing other insects present in the same environment. The writers proved to be no exception, in their five years observations on the egg-clusters of *Diatraea saccharalis* F., for they inevitably did notice other insects present in the fields of young cane which were being examined. To be sure, the record cards had no particular space reserved for notes on other insects, but they were large enough so that plenty of space could be found, on the back, if not on the front, for writing down all sorts of notes, some with no entomological bearing. Many a card bears speedometer reading of actual miles driven, or a note on whether the field had been previously examined, or a rough field map of its exact position, or sometimes such a record as "Dry as Hell", or "Game called on account of rain."

Of those with the most direct bearing on the project are the rare records of finding an adult *Diatraea* moth in the field. At first also, the surprise of finding adult *Trichogramma* on egg-clusters resulted in a record of each such instance, but soon this ceased to be surprising, and such notes no longer appear, altho instances of finding the minute parasites in the immensity of a cane field were as common as before. It is believed, however, that all instances of finding the much less common *Prophanurus alecto* Crawford, in the field on egg-clusters, or emerging from collected egg-clusters, were recorded. Naturally, also, every instance of finding the little black ant, *Monomorium carbonarium ebeninum* Forel, eating egg-clusters was incorporated in our field notes, for this was an entirely new aspect of its presence in cane fields (Wolcott & Martorell 1937), which actually resulted in the destruction of a sixth of all *Diatraea* egg-clusters. All such notes have been included in our report on the main

project; the purpose of the present paper is to summarize all the other entomological notes, which as here consolidated, form the entomological aspect of an ecological survey of fields of young cane in Puerto Rico.

No originality is claimed for the idea of such a survey, for soon after the initiation of the Sugar Cane Producers' Experiment Station at Rio Piedras, Mr. D. L. Van Dine (1913) issued an annotated list of the insects of sugar-cane in Puerto Rico. The senior writer (Wolcott 1921) made a survey from December 1920 to July 1921 of the insects which might be implicated in the transmission of mosaic disease of sugar-cane. The observations here reported, cover a longer period (five years) than any of the others, and give the present status of the insects concerned.

#### MAY BEETLES

How different is the present status of some of these insects may be judged from the fact that the founding of the Sugar Producers' Station was primarily due to the need for finding some practical method of controlling white grubs in cane fields. The story of the introduction of the giant Surinam toad, *Bufo marinus* L., (Fig. 1 & 2) has already been

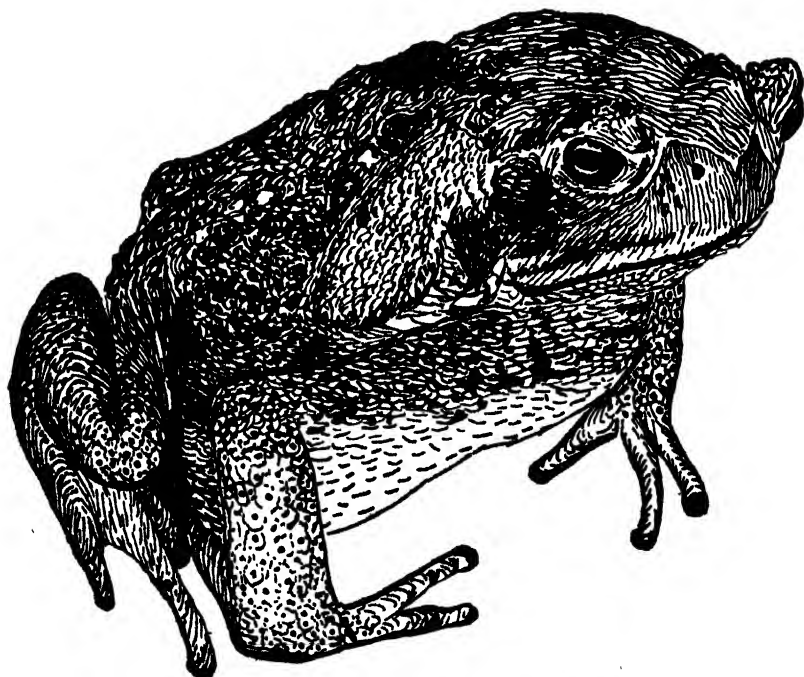


Fig. 1. The Giant Surinam Toad, *Bufo marinus* L., adult female, natural size. (Original, drawn by G. N. Wolcott).

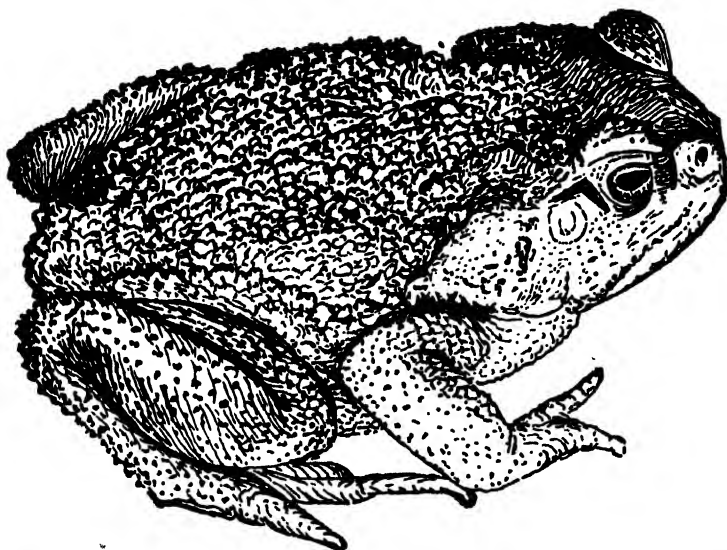


Fig. 2. The Giant Surinam Toad, *Bufo marinus* L., adult male, natural size. (Original, drawn by G. N. Wolcott).

told several times (Leonard 1933, Wolcott 1935), but to one who remembers the conditions previous to its becoming abundant and omnipresent in Puerto Rico, the change caused by it in the status of white grubs seems almost incredible. *Bufo* was no respecter of the specific identity of May beetles, and ate the species endemic and peculiar to Puerto Rico (Fig. 3) as readily as any of those of continental South America, or those of any country into which it has since been successfully introduced. Its environmental requirements coincide with those of the most destructive species of Puerto Rican May beetles, and when these are not available for food, eats other large insects, or millipedes, or, failing any of these, uncomplainingly retreating into shallow sheltering caves beneath clods of earth, quietly fasts until May beetles are again available. For a few years after the toads had become most abundant, they were so completely successful in the control of white grubs (Fig. 4) that an insufficient supply of food was available to maintain them in such great numbers. A more stable equilibrium has by now been established, so that while an abundance of toads promptly appears whenever an exceptional food supply develops, few are to be noted where there is little for them to eat. Localized outbreaks of May beetles still occur, and in our notes are thirteen records of cane shoots being half defoliated during April and May, one record at Arecibo in June and another at Humacao in August. In every case, pellets of toad excrement showed how



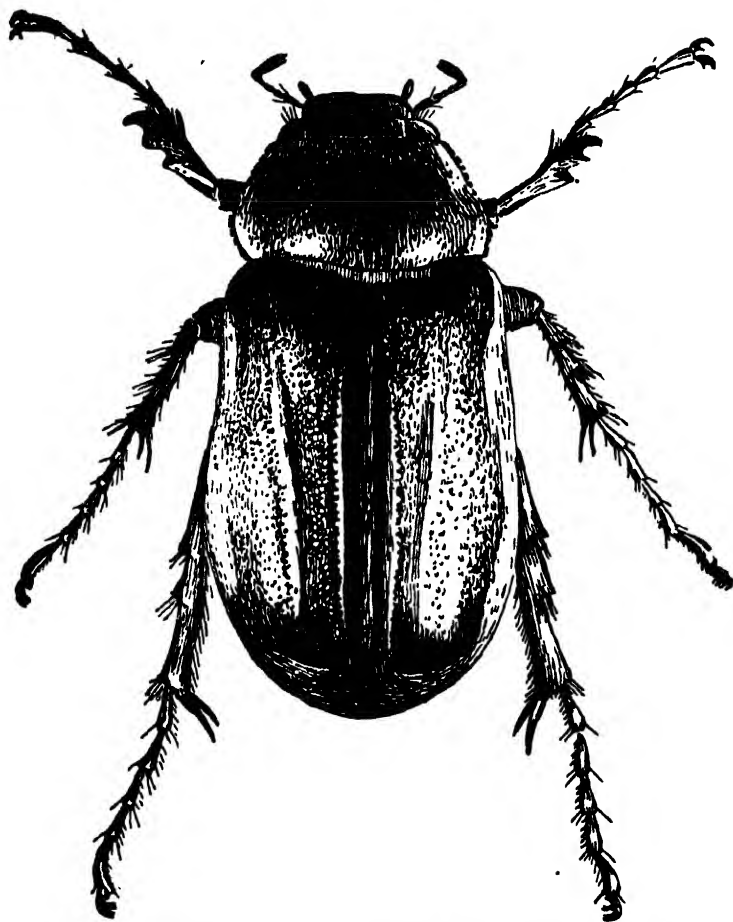
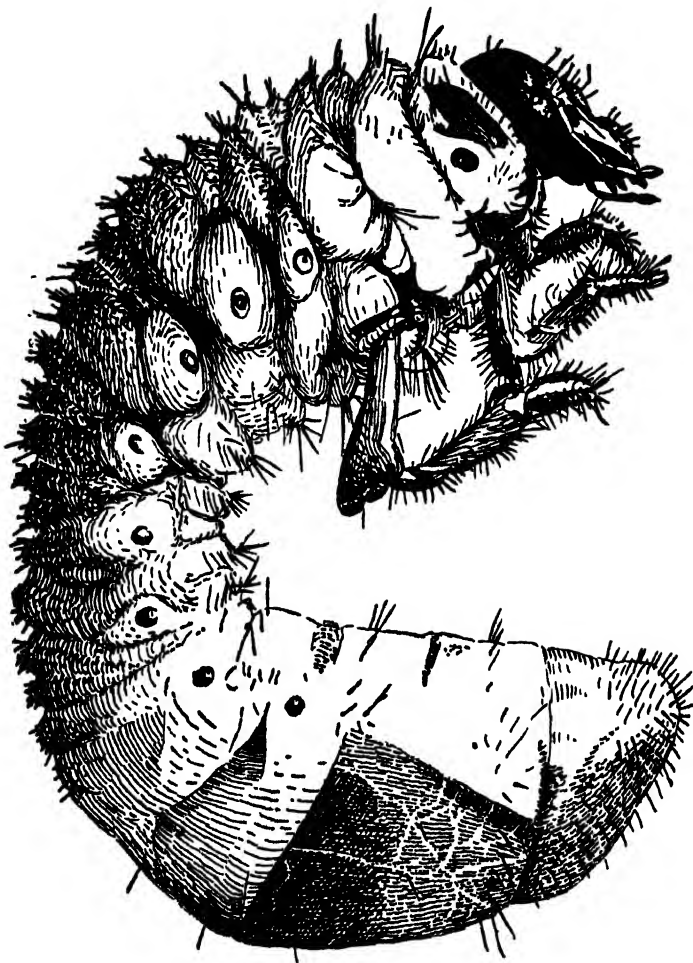


Fig. 3. The common Puerto Rican May Beetle, *Cnemerachis* (*Lachnosterna* or *Phyllophaga*) *portoricensis* Smyth, three times natural size. (Original, drawn by G. N. Wolcott).

temporary in character were such outbreaks, as the toads of the region had promptly mobilized where food was abundant.

#### SOUTHERN GRASS WORM

The black "hard-back" Lamellicorn beetles, the large leaf-eating Otiorhynchid weevils or "vaquitas", and millipedes are the other largest fractions of the food eaten by toads, for their size, slowness of movement, and habits render them the most easily captured, and their abundance makes them most available. *Bufo* neves misses a chance, however, to feed on any-



**Fig. 4.** The common Puerto Rican White Grub, *Cnemerachis* (*Lachnosterna* or *Phyllophaga*) *portoricensis* Sm th, five times natural size.  
(Original, drawn by G. N. Wolcott)

thing of reasonable size, and altho caterpillars consist mostly of undigested chips of leaves not yet transformed from vegetation into insect, even they are not despised by the toads. Outbreaks of the southern grass worm, *Laphygma frugiperda* S. & A., (Fig. 5) are no longer as severe as before *Bufo* was introduced, and toad excrement in a field where these caterpillars are abundant consists largely of telescoped caterpillar skins and the skulls of *Laphygma* still readily identified by the characteristic inverted Y. All

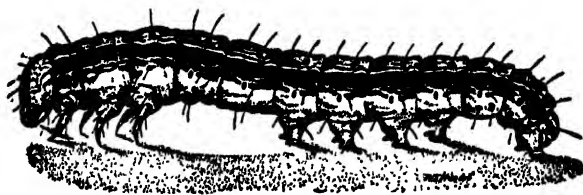


Fig. 5. Larva of *Laphygma frugiperda* S. & A., twice natural size. (Drawn by W. R. Walton).

the other parasites of *Laphygma* noted by Jones (1913) are also present: the Tachinid flies hovering about, the cocoons of *Apanteles marginiventris* Cresson on cane leaves, the adults of *Chelonus insularis* Cresson looking for egg-masses to parasitize, and very rarely a mummied caterpillar with cocoons of *Euplectrus* beneath its shrivelled remains. Of course *Bufo* eats the parasitized caterpillars as readily as those which have escaped their numerous enemies, and might readily eat the parasites also, if they were not too quick-moving to be caught. In the complex of competitive forces its presence is thus not exclusively beneficial to the interests of the cane grower, but the total effect seems to be that rarely does an outbreak last for more than a single generation of caterpillars. Our notes concerning fields which were observed a number of times are of complete recovery

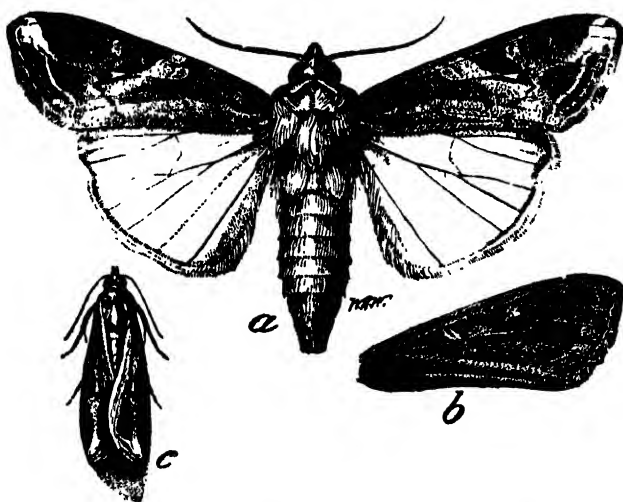


Fig. 6. *Laphygma frugiperda* S. & A., adult with folded wings (c) is natural size. (Drawn by W. R. Walton)

by the following observation, usually a month later, accompanied by an abundance of parasites with nothing to attack, and of pellets of toad excrement mostly composed of caterpillar skins.

As a pest of sugar-cane, *Laphygma frugiperda* (Fig. 6), is usually considered of only minor importance, because outbreaks are so promptly outgrown and leave no trace in the mature crop. Its actual status is more serious, for the cane crop has only so many months in which to reach maturity, and if one or more months is sacrificed at the beginning to make up for what *Laphygma* caterpillars have eaten, the injury in just that fraction of the growth period. Indeed, our search for the egg-clusters of *Diatraea saccharalis* was so often rewarded with much larger and more easily seen egg-clusters of *Laphygma* (Fig. 7), as to suggest that the hand-

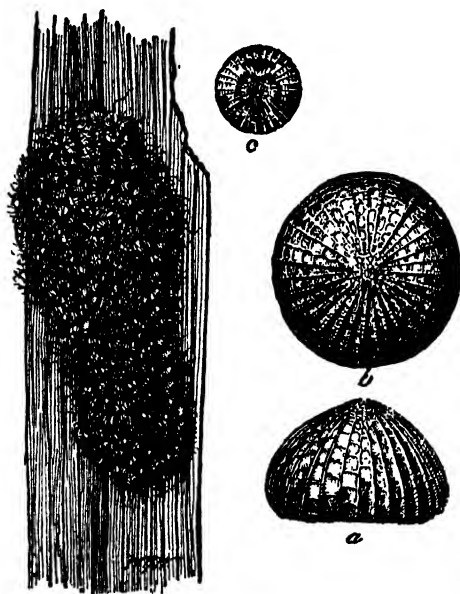


Fig. 7. Eggs of *Laphygma frugiperda* S. & A. Egg mass at left about twice natural size: (a) highly magnified egg, side view; (b) same from above; (c) egg ready to hatch, larva showing thru the shell. (Drawn by W. R. Walton).

collection of *Laphygma* egg-clusters would be an inexpensive method of protecting young gran cultura cane from its attack. During five years observations in June, not a single *Laphygma* egg-cluster was noted, but in July and August records begin to appear and of first injury by cater-

pillars, going up rapidly in number and intensity in September and reaching a peak of fifteen records of severe injury in October. We have few records for November, but again a considerable increase in December, and scattered records for the remainder of the year. Since it is thus possible to predict with reasonable assurance that injury is most likely to occur in September and October, hand-collection of egg-clusters in August and September might prevent outbreaks. Indeed, at least a preliminary inspection of all young plant cane at any time of year would appear to be justified.

#### MOCIS REPANDA

The looper caterpillar, or "argrimensor", *Mocis repanda* F., is quite as destructive as *Laphygma*, but rarely so abundant (Jones & Wolcott 1922). It follows the same seasonal pattern of abundance: our records of injury being for October, November and December: pupae and old injury in January, one record of small caterpillars in February at Manati and another in July at La Vega, Arecibo. In some years, it may not be observed at all, our first record being in 1939, but at times in the past it has been noted to sweep a field of young plant so clean that nothing remained but the naked central shoots.

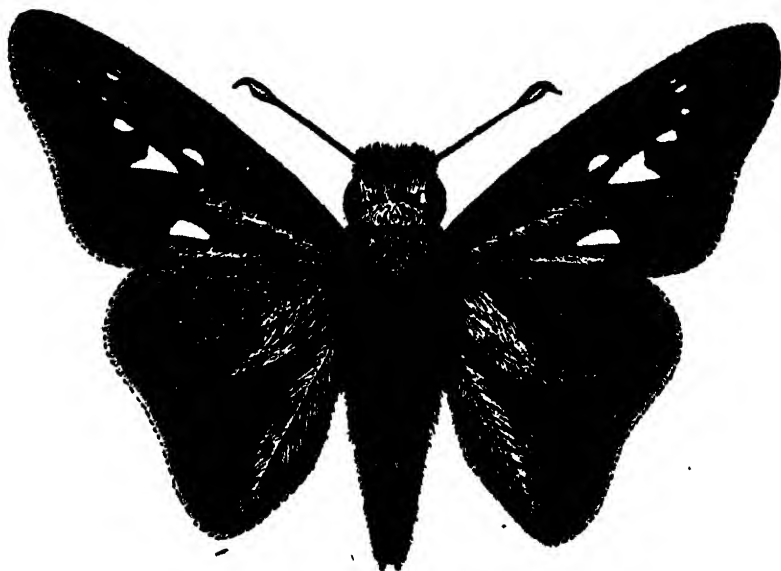


Fig. 8. *Panoquina nero* F. Twice natural size.  
(Drawn by Thos. H. Jones).

## HESPERIID BUTTERFLIES

The little brown skipper butterflies (Fig. 8), looking like minute fighter or pursuit planes with their stubby bodies and short wings, occur in cane fields because their caterpillars (Fig. 9) feed on cane leaves, or

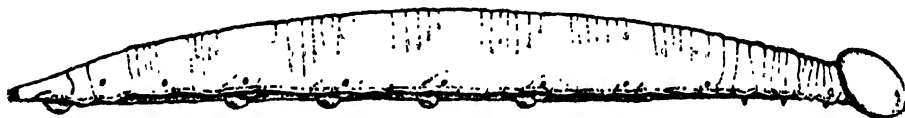


Fig. 9. Larva of *Panoquina nero* L. Twice natural size.  
(Drawn by Thos. H. Jones).

the blades of other coarse grasses. Economically they appear to be of very minor importance, but this is only because their specific egg-parasite, identified by Mr. A. B. Gahan as an undescribed species of *Ooencyrtus*, is so abundant. From October to February, when eggs are most numerous, two-thirds or more of all eggs collected are black with parasitism, and all of the smaller number of eggs during the summer are parasitized. We noted not a single caterpillar from April to September, but in October they were quite abundant at many points in eastern Puerto Rico, and in most winter months were noted repeatedly at Rio Grande. The Vespid wasp, *Polistes crinitus* Felton, was twice seen eating caterpillars. The eggs and smaller caterpillars of the more common *Panoquina (Prenes) nero nero* F. are practically indistinguishable from those of *Panoquina nyctelius coscina* Herrich-Schaffer (= *Prenes ares* Felder), and we made no effort to do so. The large Herperiid, *Perichares coridon coridon* F., is so conspicuous because of its size and hairyness that we could be sure of every record. Caterpillars or pupae were found only from September to April, and only on the north coast: Coloso to Mameyes. One fully grown caterpillar, possibly attempting to pupate, was found being eaten by crazy ants, *Prenolepis longicornis* Latreille.

## A LEAF-TYER

Ruining the appearance of young cane fields, but at present of no economic importance in Puerto Rico because of its scarcity, is a leaf-tyer. *Marasmia trapezalis* Guenée, the caterpillars of which feed on the tips of the leaves of young cane. The first record of this insect in Puerto Rico was at Barrio Camacey, Isabela, in July 1931, a single caterpillar only in a large cane field, which was reared to adult and determined by the late Dr. William Schaus as *M. similis* von Hedemann. The type of *M. similis* is from St. Croix, but in economic literature it has not been re-

corded from there as a pest of sugar-cane. Mr. Carl Heinrich states that the specimen identified by Dr. Schaus agrees in all details with typical *trapezalis*, previously recorded as a pest of sugar-cane from Hispaniola and Peru (Wolcott 1929). Our records in Puerto Rico would appear to indicate its recent arrival from Hispaniola; very abundant in Barrio Aguacate, Isabela in October 1936, as well as in another field near Camuy; also very abundant in two fields at Guanica in November 1939, and in a field at Yauco in December 1936, and one at Sabana Grande in the same months in 1937. The only other record of its presence is in July 1937 at Guayanilla. All of these localities are on or near the west coast of Puerto Rico, closest to Hispaniola.

#### SEASONAL ABUNDANCE

Our work on *Diatraea saccharalis* indicated no period of its maximum abundance general to all of Puerto Rico, yet every other caterpillar infesting sugar-cane: *Laphygma*, *Mocis*, the Hesperids and even the rare *Marasmia*, show the same seasonal reaction of greatest abundance in the late fall and early winter months. Why should *Diatraea* be the exception?

#### YELLOW APHD

Quite different from the Lepidoptera in its pattern of reaction to season is the yellow aphid of sugar-cane, *Sipha flava* Forbes, which our observations indicate as having two periods of abundance. Most of the records are for the months of December, January and February, none in March, a few for April, and again abundance in May and June, with none for the summer and only a few for autumn. Regionally, it is a much more serious pest in the extreme eastern part of Puerto Rico, three-fourths of the records of serious infestations being from Guayama and Arroyo to Fajardo and Loiza. This can hardly be a reaction to the variety of cane grown, for BH (10) 12 is as much the standard cane for Guanica and Ponce as it is for Guayama and Loiza, while Fajardo with its own special seedlings suffers no less than Yabucoa and Arroyo.

During the period, but outside the region of our observations, especially heavy and prolonged infestations occurred during the autumn of 1940 around Caguas and Juncos, which were aggravated by dry weather, and almost destroyed some fields of young gran cultura cane. Heavy rainfall in late October eliminated these infestations. The effect of the rain by continuously maintaining a high humidity for several days, was to induce an epidemic of fungus disease caused by *Acrostalagmus aphidum* Oud.

While epidemics of fungus disease are completely effective in control when, and only when suitable climatic conditions for their development occur, commercially effective control under any climatic conditions may, and often does result if the lady-beetle, *Cycloneda sanguinea* L., and others, and the Syrphid fly, *Baccha latiuscula* Loew, and others, are present in sufficient numbers. The value of these predators is considerably limited by their natural parasites, however, the natural balance thus resulting giving the aphids a chance to maintain their numbers, or at least to avoid total extermination locally. At other times, one may find large numbers of *Cycloneda sanguinea* adults wandering about in a cane field clearly showing indications of having been heavily infested by aphids not many days before, but now devoid of anything for the beetles to eat. Eventually they disperse widely by flight, but it was under such conditions that a larva, not able to get away thus easily, was noted attempting to extract nourishment from the empty egg-shells from which *Diatraea* caterpillars had hatched.

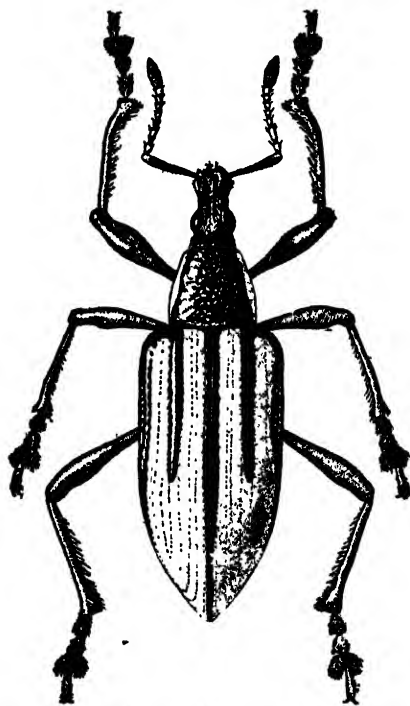


Fig. 10. *Diaprepes abbreviatus* L.  
Five times natural size.  
(Drawn by H. Bradford).



The presence of the brown aphid, *Hysteroneura setariae* Thomas, a very minor pest of sugar-cane, was noted once at Santa Isabel in November 1936, and not again recorded.

#### VAQUITA

The Otiiorhynchid beetle commonly known in Puerto Rico as "vaquita", *Diaprepes abbreviatus* L. (Fig. 10), in its relations with sugar-cane has been studied mostly as larvae burrowing into and feeding upon the root-stalk (Jones 1915). As so little is known about the eggs of the vaquita on sugar-cane in Puerto Rico, we made a definite effort to record



Fig. 11. Egg-clusters of *Diaprepes abbreviatus* L.. between leaves of jobo. Twice natural size. (Drawn by F. Seín).

each collection. Our notes, however, represent only incidental collections, for the presence of the eggs is not obvious in most cases, and was usually detected by accident. Normally they are laid in masses of considerable size between the split ends of the leaves, altho we have several records of their being laid between two crossed or adjacent cane leaves, as is typical on the leaves of trees (Fig. 11). When laid on very tender cane leaves, their presence may result in the leaf turning reddish-brown clear thru to the other side, but instances of such distinctive pigmentation are rare. A few egg-clusters were noted on sugar-cane during the spring months, many in May, reaching a peak of greatest abundance in June. Some were found in July and August, with a secondary peak in abundance in September and October, decreasing greatly in abundance thereafter, so that none was found after early December. At Guanica, Guayanilla and Ponce, eggs were found only in the months of peak abundance of June and September, all the records for other months being at localities in the more humid parts of the Island.

One might suppose that abundance of eggs parallels abundance of adults, but the adults feed by preference on so many other hosts that occurrence of eggs on cane does not necessarily imply parallel abundance of adults. One never finds hundreds and thousands of adults on cane, as is common on preferred individual host trees, but usually only an occasional individual, or rarely one or more pairs close together. While the grubs feed on the root-stalks of sugar-cane to such an extent as to be a major pest, adults rarely eat cane leaves, at least in Puerto Rico, greatly preferring the leaves of trees bordering cane fields. We have two definite records of *Diaprepes* adults eating tender cane leaves, however: at Isabela in March and at Naguabo in May, both recorded because such occurrences are so exceptional.

The specific parasite of *Diaprepes* eggs, *Tetrastichus haitiensis* Gahan, has the same peak of abundance in June, but normally attacks eggs laid between leaves that are less tough than the drying tips of those of sugar-cane. Indeed, the failure of this parasite to survive in Barbados was thought by Mr. R. W. E. Tucker (1936) to be due to its inability to penetrate cane leaves, either for oviposition in the host eggs, or for emergence from them. Our notes record four instances of parasitism by *Tetrastichus* of vaquita eggs laid between cane leaves: two instances in May, at Luquillo and Rio Blanco, one in August at Isabela and another in December at Camuy. Quite possibly, however, these are such exceptional occurrences as to be immaterial in beneficially affecting the possibility

of survival of the parasite in the hostile Barbadian environment. Indeed, they represent less than 5% of all egg-clusters collected, quite insignificant by comparison with almost total parasitism of *Diaprepes* egg-clusters laid between citrus or jagüey leaves in June.

#### THONALMUS

The work of the economic entomologist so often deals with some foreign pest, accidentally introduced, that it is with the greatest pleasure that one can record the accidental introduction and establishment of an insect which not only is not a pest but a beautiful addition to the local fauna: *Thonalmus dominicensis* Bourgeois.

The Lesser Antilles to the east and south of Puerto Rico are in general less rich in insect life than is Puerto Rico, and it is doubtful if many introductions, accidental or intentional, have come from this direction. Indeed, the only one of which we are at all certain, in recent times, is of the large Vespid wasp, *Polistes major* P. B., presumably blown in by the hurricane of 1928 (Bequaert 1936), and possibly one other, the pepper flower bud moth, *Gnorimoschema gudmanella* Walsingham, from the Virgin Islands. The much larger island of Hispaniola to the west, inhabited by many insects not found in Puerto Rico, is to the leeward both as to normal trade winds and hurricanes. Inter-island traffic, however, tends to bring numerous insects to Puerto Rico from Santo Domingo, not only in mahogany sawn lumber ("la bete d'argent", *Polycesta porcata* F., has emerged from furniture made in Puerto Rico of lumber imported from the Dominican Republic), but in unbarked railroad ties, and in cargoes of sugar-cane. To be sure, all the cane boats daily or tri-weekly bringing cane from La Romana to Ensenada during the grinding season are fumigated with sulfur, which actually has prevented the Hispaniolan cane butterfly, *Calisto pulchella* Lathy, from gaining entrance, or at least from becoming established in Puerto Rico.

When inspection of this fumigation was initiated by entomologists, it was common practice to wait until the boat was unloaded and see what dead insects could be collected in the empty hold. Among others was the bright red and blue Lycid beetle, *Thonalmus chevrolati* Bourgeois, first recorded by R. H. Van Zwaluwenburg from Puerto Rico and doubted by Leng & Mutchler (1922). Nevertheless, Van Zwaluwenburg was right as to the presence of live beetles in Puerto Rico and of their establishment here, for whether they survived the sulfur fumigation, or arrived in Puerto Rico by some other means, the first records of their presence

in cane fields are at Guanica (Wolcott & Martorell 1937a). We first noted the live beetles in young cane near Yauco in March 1937, at Guayanilla in June and September, and at Guanica in September and November. In 1938, beetles were noted only at Guanica, but in 1939 were abundant in March at Guanica, Guayanilla and Tallaboa, and again in midsummer and in the autumn at all of these points.

In March 1940, a single beetle was collected at Hda. Victoria, between Coloso and Aguadilla, and several were noted in the same or adjacent fields in April and July. The only records for 1941 are of collections in Ponce in May and June, possibly because records at former points of collection were no longer of primary interest. Unquestionably, all of the records at Guanica, Yauco, Guayanilla, Tallaboa and Ponce indicate a consistent dispersion along the south coast from the first introduction at Ensenada harbor, but this by no means accounts for the records at Coloso, an entirely different ecological region and one towards which no traffic occurs likely to carry the beetles from the south coast. The late Dr. Stuart T. Danforth records under the name *Thonalmus dominicensis* Chevrolat (Wolcott 1936), collection of this beetle at Hormigueros in August 1932 by Felipe Mora, to that extent at least filling in the gap between the pier at Ensenada and the cane fields of Coloso, and indicating dispersion up the west coast from the port of entry. A more intimate knowledge of the life-history of *Thonalmus* would be most desirable, for we can see no specific or necessary connection with sugar-cane. Of course, all of our records are from fields of sugar-cane, but with an equal amount of attention given to other environments, its relative abundance might prove to be much greater outside of cane fields, or its occurrence in them be due merely to the fact that they are humid, irrigated areas. Its dispersion to Coloso, against traffic in mature cane, all of which is going in the opposite direction towards Guanica Centrale, might indicate dispersion by flight, and possibly to a preferred environment in a more humid region, for the distance to Coloso is twice as great as to Ponce.

That we have not found this beetle on Mona Island, between Puerto Rico and Hispaniola, may be due to the xerophytic character of Mona, or it may be additional evidence of the probability of the introduction of *Thonalmus* into Puerto Rico by commerce, for there is little or none from Hispaniola to Mona Island.

#### TWICE-STABBED LADY-BEETLE

Another recently introduced beetle, present also only by accident in cane fields, is the twice-stabbed lady-beetle, *Chilocorus cacti* L., noted

twice near Manatí in 1940 and in 1941. Presumably these are descended from adults released at Vega Baja and Barceloneta by the Mayaguez Station (Anon. 1939) as predators on the scales of bamboo. In the cane fields where noted, they were far from any scale-infested bamboo, but these lady-beetles feed on a great variety of scale insects on many different hosts. At Rio Piedras, they became very abundant on papaya and acalypha, feeding on the grey scale, *Pseudoparlitoria ostreata* Cockerell; on coconut feeding on *Aspidiotus destructor* Signoret, and on emajagua feeding on *Pinnapis minor* Maskell. At Isabela they have cleaned citrus trees of heavy infestations of *Chionaspis citri* Comstock; in the Maricao Insular Forest been observed feeding on scales on *Trema lamarchiana*; at Guanica feeding on *Asterolecanium pustulans* Cockerell on *Colubrina colubrina*, and most recently been recorded from Cabo Rojo feeding on *Saissetia hemispherica* Targioni on pigeon peas. It is hardly surprising, therefore, that these beetles wandering from hosts which they have cleaned of scales should now and then be found in cane fields, even if there is nothing in particular for them to eat there.

#### OTHER INSECTS

Several of the other insects noted in cane fields fill a specific niche in the environment where they were found, expressing a reaction to some particular factor in that environment. As a reaction to the burning of trash, numerous dead-hearts caused by the caterpillars of *Elasmopalpus lignosellus* Zeller were noted in a field near Toa Baja in June 1937. During dry weather, one often sees a few chinch bugs, *Blissus leucopterus* Say, in the cane fields, the one record we have of considerable abundance being at Rio Grande in October 1937. Cane planted on sandy land is often attacked by the changa, *Scapteriscus vicinus* Scudder (Fig. 12), such injury being so common that unless serious, we made no note of it. The records of worst injury are at Hda. San Isidoro near Patillas, at Santana near Arecibo, and Hda. Maria Teresa at Camuy. Another insect often noted in sandy land, but only indirectly connected with cane, is the Scoliid wasp, *Campsomeris dorsata* F., repeatedly noted in some sandy fields at Guanica in the winters of 1938 and 1939, and on red sandy land at Manatí. A large cluster of males was noted at Patillas in July before the middle of the afternoon, and many females frequenting the flowers of "verdolaga", *Portulaca oleracea*, at Guayanilla before 10 o'clock in the morning.

An abundance of the large black Stratiomyid fly, *Hermetia illucens*





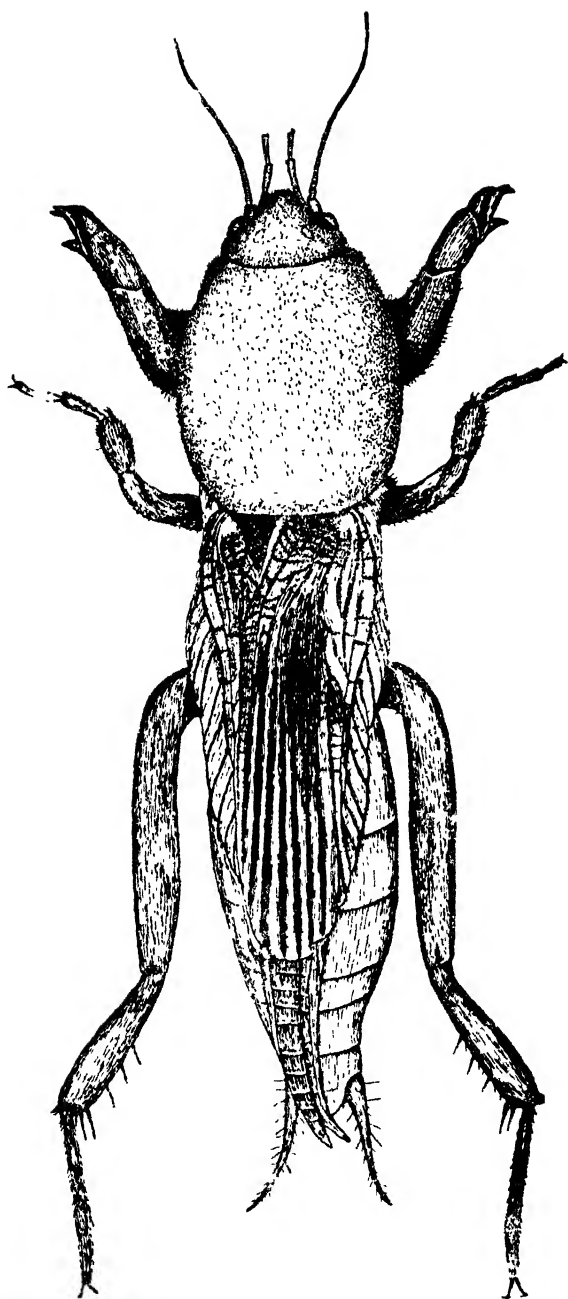


Fig 12. The "Chunga" of Puerto Rican Mole Cricket, *Scapteriscus vicinus* Scudder, Six times natural size (Drawn by F. Seft)



L., presumably indicates heavy fertilization with organic manure or cachaza, or the presence near-by of a pile of such decaying organic material, on which the larvae are known to subsist. A few flies were often seen, but the only record of exceptional abundance is at Hda. Las Claras near Arecibo, in July 1939, just before noon. The presence of *Euxesta thomae* Loew in cane fields, was just a nuisance from our standpoint, because at first glance they often looked so much like parasitized or partly parasitized *Diatraea* egg-clusters, and in consequence they received not a single written record from us. *Grallopoda* (*Calobata*) *lasciva* F., another fly common in cane fields, was recorded only when numerous dead adults, stuck to the leaves by a white fungus, were noted at Canovanas in November 1936 and at Guayanilla in December 1938.

#### GRASSHOPPERS

Cane grown in other countries adjacent to extensive uncultivated semi-desert regions often suffers severely from invasions of grasshoppers, but in Puerto Rico grasshoppers are rarely abundant in any environment, and have never been recorded as causing appreciable injury to cane. They do feed on the leaves of sugar-cane, nevertheless, and in a field of cane near Fajardo, now a housing development, little green *Schistocerca* nymphs were observed in November 1938, and at each succeeding observation were noted growing rapidly, had transformed to adults by February, which were still present in March when the cane became too high for further observations. A single large green grasshopper, *Neoconocephalus triops* L., was observed feeding on a cane leaf at Camuy, and the wasp *Tachytes insularis* Cresson was noted carrying a smaller green grasshopper, *Conocephalus cinereus* Thunberg, to provision her nest, in a cane field at Toa Baja.

#### A NEW MITE

Now that every cane grower, and nearly every laborer in the cane fields of Puerto Rico knows and recognizes mosaic disease, the simulation of this disease by the attack of multitudes of a little green mite living on the underside of cane leaves is becoming increasingly common. Mr. E. A. McGregor, a specialist in mites of the genus *Tetranychus*, has described it as new, with the type from Puerto Rico. Our notes fail to give all the records, but it was abundant in a field of M-28 near the new hospital at the top of the hill near Aguadilla, and noted on this variety

of cane at several other points, as well as on standard varieties at Vega Baja in May 1940, at Quebradillas in July, and in great abundance in several fields near Arecibo in August 1941. The mites were first noted infesting sorghum growing in a greenhouse at Rio Piedras early in 1940. Possibly the heaviest infestation noted was at Loiza in August 1941 on BH (10) 12, where the mites were being attacked by small yellowish dipterous maggots, as well as by the larvae of very small black lady-beetles, identified by Dr. E. A. Chapin as a species of *Stethorus*. Actual injury by these mites has been negligible up to the present, but it is fortunate that checks on their abundance have already developed, and presumably will prevent this new pest from becoming more than a possible threat.

#### SUMMARY

Five years observations in fields of young cane in Puerto Rico indicate a marked seasonal abundance during autumn and early winter, of all caterpillars except those of *Diatraea saccharalis* F., the species noted being *Laphygma frugiperda* S. & A., *Mocis repanda* F., *Panoquina n. nero* F., *P. coscina* Herrich-Schäffer, *Perichares c. coridon* F. and *Marasmia trapezalis* Guenée. The yellow aphid, *Sipha flava* Forbes, is most abundant during the winter and again in late spring, especially in eastern Puerto Rico. The eggs of *Diaprepes abbreviatus* L. are most abundant in June and September, and on the south coast were found only during these months. *Thonalmus chevrolati* Bourgeois, a red and blue Lycid beetle accidentally established at Guanica by introduction from Santo Domingo, has spread north to Coloso and east to Ponce. The twice-stabbed lady-beetle, *Chilocorus cacti* L., introduced to prey upon the scales attacking bamboo, feeds on many other kinds of scales, and at times is found in cane fields. The injury produced by a new species of mite, *Tetranychus sacchari* McGregor, simulates mosaic disease.

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## RELATION OF MOISTURE CONTENT TO QUALITY OF VANILLA BEANS

by

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### INTRODUCTION

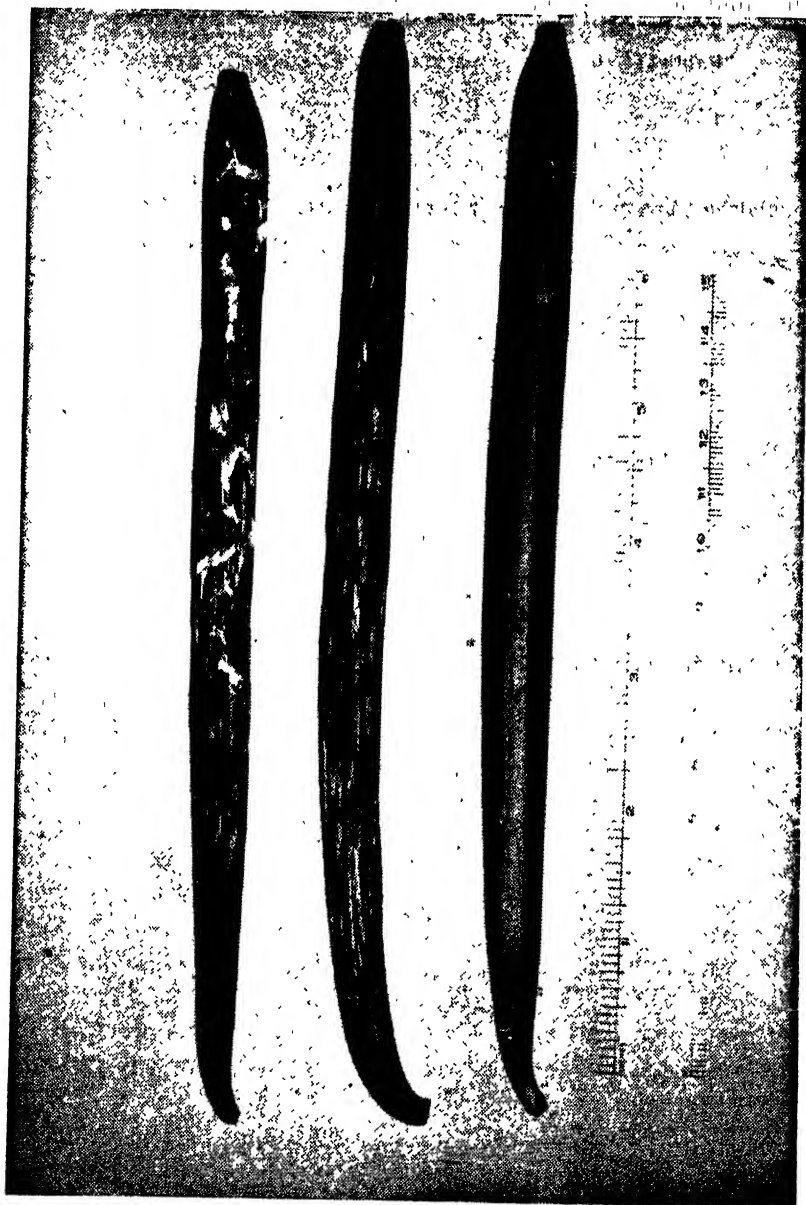
Vanilla beans undergo during curing a series of chemical transformations which lead to the formation of flavoring compounds. (1, 3). Curing consists usually (5, 6, pp. 84-117, 7) of an initial killing or wilting treatment which arrests the natural vegetative processes and hastens chemical changes. After this treatment, the beans are usually subjected to heating and subsequent sweating in blankets or else continuous heating. The process is completed by further partial drying at room temperature and finally aging or conditioning in wooden boxes.

Curing is accompanied by a loss of moisture which starts during sweating and continues throughout the drying and conditioning periods. The moisture left in the beans influences their appearance and flexibility. A relatively high percentage of moisture is desirable and produces higher returns as the beans are sold by weight. It is of importance, therefore, that the curer of vanilla controls moisture losses during curing.

Figure 1 shows some of the visible differences between freshly harvested and cured beans of *Vanilla fragrans* (Salisb.) Ames, the vanilla of commerce.

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**Figure 1.** Vanilla beans; one at left, freshly harvested and two at right, cured. The white marks on the surface of the cured bean at the extreme right are crystals of vanillin, the most widely known of the aromatic constituents of vanilla.

## MOISTURE CONTENT OF UNCURED BEANS

Maturation is indicated in uncured vanilla beans by the development of a yellow coloration at the blossom-end. As ripening increases, this coloration spreads along the entire bean and is often accompanied by longitudinal splitting. Fully mature beans finally acquire a chocolate color.

Analyses were made to determine variations in moisture content of beans of different maturities. Beans 6.5 to 7.5 inches in length, harvested about 5 days previously, were graded according to maturity as whole beans, entirely green; whole, blossom-end yellow; split, blossom-end yellow; and split, entirely chocolate. From each grade of beans three random samples were selected. Moisture determinations were made by vacuum oven-drying at 68° to 70° C. at 100 mm. pressure. (2, p. 336)

Whole beans, entirely green were found to have an average moisture content of 81.2 percent, whole beans with blossom-ends yellow 79.2; split beans with blossom-ends yellow 79.0, and split beans all chocolate 73.5. The moisture content of split beans varies to a large degree with the extent and time of splitting. The percentage of moisture in the beans thus decreased with maturity.

## LOSSES IN WEIGHT DURING CURING

A study was made of vanilla beans subjected to different killing treatments to determine whether a significant weight loss other than that due to loss of water occurs during the chemical transformations which take place during curing.

The beans used were 6.5 to 7.5 inches long and were at different stages of maturity. The treatments consisted of freezing for 4 and 20-hour periods, three 10-second immersions at 30-second intervals in water heated to 80° C., exposure to ethylene gas for different periods at a concentration of 1 part to 10,000 parts of air, rubbing with alcohol, exposure to the sun, and placing in an oven at 65° C. These treatments, which were applied singly and in combination, were followed by sweating in an oven or in the sun, further drying on racks at room temperature to the desired loss in weight, and conditioning in closed wooden boxes for 3 months. The loss in weight of the beans during curing was recorded and the amount of moisture in the final, cured beans was determined. The results classified according to maturities and the killing treatments used, are shown in table 1.



Table 1. Loss in weight and moisture content of vanilla beans of five stages of maturity during curing after various killing treatments (1)

WHOLE BEANS, BLOSSOM-END GREEN

<i>Killing treatment</i>	<i>Total loss in weight during curing</i>	<i>Moisture content of cured beans</i>	<i>Loss in weight during curing plus moisture left in cured beans (%)</i>
	PERCENT	PERCENT	PERCENT
Frozen for 4 hours -----	74.2	26.6	81.1
Frozen for 20 hours -----	72.7	28.8	80.6
Frozen for 4 hours; hot water -----	72.8	26.8	80.1
Hot water; frozen for 4 hours -----	72.5	26.5	79.8
Hot water -----	75.6	20.4	80.6
Rubbed with alcohol; hot water ----	72.2	26.9	79.7
Ethylene gas for 4 14-hour periods----	76.0	20.3	80.9
Ethylene gas for 14 hours -----	75.1	19.8	80.0

WHOLE BEANS, BLOSSOM-END YELLOW

Frozen for 4 hours -----	72.9	27.9	80.5
Frozen for 20 hours -----	70.2	27.8	79.0
Frozen for 4 hours; hot water -----	72.3	26.1	79.5
Hot water; frozen for 4 hours -----	72.1	28.5	80.1
Hot water -----	75.6	20.7	80.7
Rubbed with alcohol; hot water -----	71.4	26.7	79.0
Ethylene gas for 4 14-hours periods ----	74.9	21.3	80.3
Ethylene gas for 14 hours -----	74.1	20.1	80.0

SPLIT BEANS, BLOSSOM-END YELLOW

Ethylene gas for 4 14-hour periods -----	73.4	20.8	78.9
Ethylene gas for 14 hours -----	73.6	21.1	79.2

SPLIT BEANS, BLOSSOM-END CHOCOLATE

Hot water -----	70.2	20.6	76.3
Ethylene gas for 4 14-hour periods ----	73.7	20.4	79.1
Ethylene gas for 14 hours -----	71.4	21.7	77.6

SPLIT BEANS, ALL CHOCOLATE

Sun -----	68.8	19.6	74.9
Oven at 65° C. -----	69.4	19.8	75.5

- (1) Three- to 4-pound lots of beans were used in each treatment, except in split beans, all chocolate, in which 2-pound lots were used.
- (2) Calculated by adding the total loss in weight during curing to the water present in the remaining percent weight of cured beans. These figures represent approximately the average moisture content of these types of uncured beans. Thus weight loss in curing, other than that due to loss of water, was negligible.

It can be observed in table 1 that regardless of the killing treatment and the final moisture content of the cured beans, the total loss in weight during curing plus the water present in the cured product was from 79.7 to 81.1 percent of the original weight for whole green beans, and from 79.0 to 80.7 percent of the original weight for blossom-end-yellow beans. These figures represent approximately the average moisture content of these types of uncured beans, as stated previously. In the blossom-end chocolate split beans the values for the losses in weight during curing plus the moisture of the cured beans showed greater variation. However, this is to be expected inasmuch as the extent of splitting and the degree of maturation of these types cause appreciable variations in the moisture content. Therefore, it can be concluded that weight loss, other than that due to loss of water, was negligible.

LOSS IN WEIGHT DURING CONDITIONING

Moisture losses in curing vanilla occur principally during the sweating and drying treatments. Weight losses also occur during the conditioning or

Table 2. Losses in weight on a fresh basis in vanilla beans during the conditioning period (1)

<i>At the end of drying</i>	<i>During the first 3-month period of conditioning (2)</i>	<i>During the second 3-month period of conditioning (2)</i>
PERCENT	PERCENT	PERCENT
75.8	- 0.2	- 0.2
75.3	.2	.1
72.3	.8	- .3
72.2	1.7	.3
72.1	.1	.0
71.2	1.2	.1
70.9	2.0	- .4
70.5	3.1	.6
70.4	2.3	- .4
70.3	2.5	.4
70.0	1.7	.6
69.8	2.7	- .2
69.8	1.9	.3
69.4	3.5	.6
69.4	2.0	.3
69.3	1.8	.3
68.9	2.9	.9
67.8	4.5	1.1
66.6	3.4	.9
63.3	6.7	1.0
62.0	5.9	1.3

(1) Three-pound lots of whole beans with blossom-end either green or yellow were used.

(2) A negative loss of weight signifies an actual gain.

final phase of the processing during which vanillin crystallizes on the surface of the beans and the characteristic vanilla aroma gradually develops.

The moisture losses during the conditioning treatment in 21 3-pound lots of beans were determined. These samples had lost from 62.0 to 75.8 percent of their weight at the end of the drying period. The beans were weighed before being placed in the conditioning boxes and at the end of 3 and 6 months. The data are listed in table 2.

As shown in table 2, there was an appreciable loss in weight in most of the lots during the first 3 months of conditioning, ranging from a gain of 0.2 to a loss of 6.7 percent. The loss in weight was negligible, however, during the last 3 months not reaching in any case more than 1.3 percent. It is apparent that moisture content approached an equilibrium during conditioning and that the loss in weight during this period was generally greatest in the beans containing the most moisture.

#### NOMOGRAPH FOR MOISTURE CALCULATIONS

A nomograph is given in Figure 2 showing the weights to which 100-pound lots of beans of different moisture contents should be reduced during curing to obtain a predetermined moisture content in the end-product.

Example: To cure 100 pounds of green beans to a desired final moisture of 30 percent, assuming or knowing 80 percent moisture content of fresh beans, run a straight line from point 80 on left axis to point 30 on right inclined axis. Read off on center axis final weight of beans after curing, including conditioning. To obtain the weight to which the beans should be dried before conditioning, the loss during conditioning should be added to that obtained from the chart. The loss during conditioning depends principally upon the moisture content of the beans at the beginning of conditioning, the number and length of times the beans are examined, and the amount of wiping to remove molds. However, for approximate purposes, the following losses may be assumed: For 30-40 percent final moisture in the cured product, 4-7 pounds of 100 pounds fresh and for 20 to 30 percent moisture, 1-3 pounds.

#### EVALUATION OF CURED BEANS WITH DIFFERENT MOISTURE CONTENTS

An experiment was conducted to determine the variation in physical characteristics, phenol value including vanillin (4), and aroma of vanilla beans cured by different procedures to varying moisture contents. Data are shown in table 3.

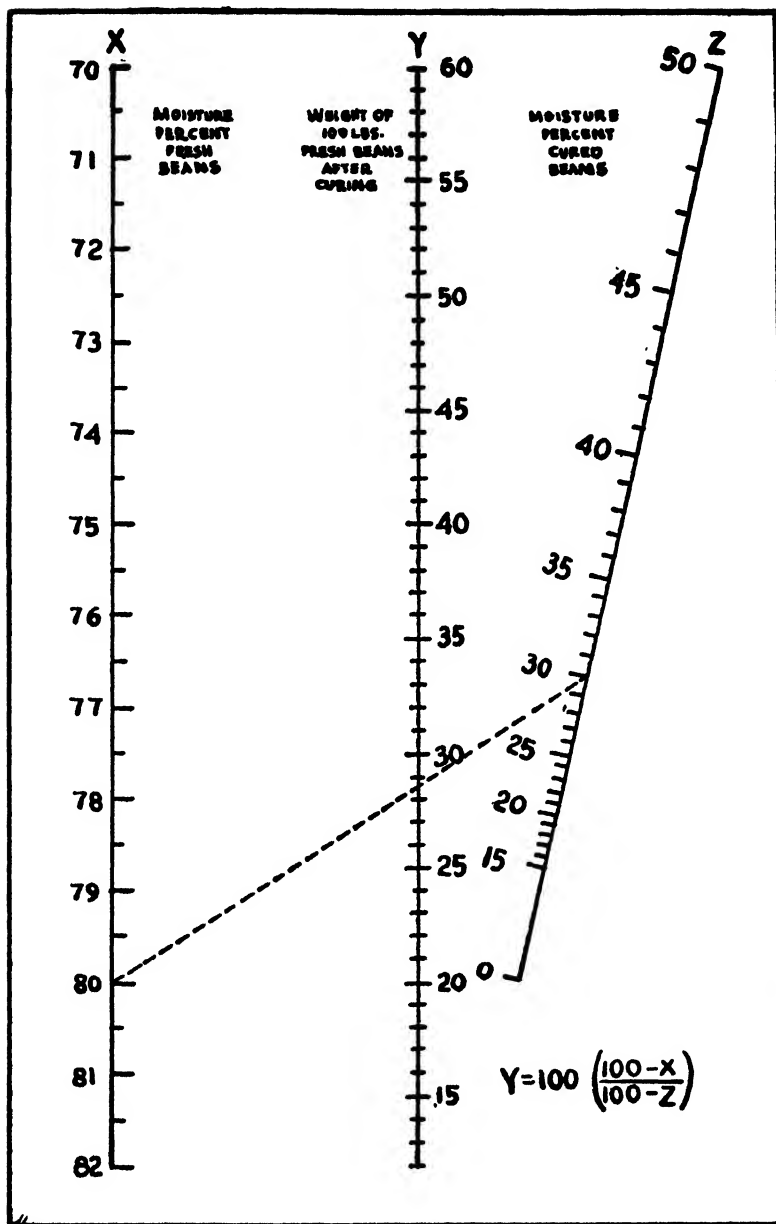


Figure 2. Nomograph relating the three variables: X=moisture content of fresh vanilla beans in percent fresh basis, Y=weight to which each 100 lbs. should be reduced during curing, and Z=moisture content of cured beans in percent cured weight basis. See example given in the text.

Table 3 Evaluation of vanilla beans cured to different moisture contents (1)

Killing treatment (2)	Final moisture content	Chocolate color	Degree of flexibility	Vanillin crystals	Mold development (3)		Phenol value dry basis	Aroma
					PERCENT	PERCENT		
Hot water	26.5	Dark	Medium	Few	0.0	6.3	6.3	Developed, suave
Hot water	30.7	Dark	Very	None	0.0	6.3	6.3	Developed, not suave
Hot water	49.9	Light	Very	None	0.0	6.4	6.4	Crude, slightly fermented, not suave
Freezing	26.2	Reddish	Very	Some	0.0	6.8	6.8	More developed, suave
Freezing	32.6	Reddish	Very	None	0.0	6.7	6.7	Developed, very suave
Freezing	50.5	Light	Very	None	0.0	6.9	6.9	Developed, slightly fermented, suave
Sun	26.5	Dark	Medium	None	0.0	6.7	6.7	More developed, suave
Sun	30.9	Light	Very	None	4.0	6.3	6.3	Developed, suave
Sun	53.0	Light	Very	None	7.0	6.9	6.9	More developed, slightly fermented, not suave
Ethylene	24.2	Dark	Little	Few	0.0	7.3	7.3	More developed, suave
Ethylene	33.6	Light	Medium	Few	3.0	7.5	7.5	More developed, suave
Ethylene	54.3	Light	Very	None	3.0	6.9	6.9	Developed, slightly fermented, not suave

(1) Beans killed by hot water and freezing were obtained from the same batch and beans killed by sun and ethylene from another batch.

(2) Beans killed by hot water, freezing and ethylene were oven-sweated and beans killed in the sun were also sun-sweated. All the lots were then dried further at room temperature to the desired loss in weight and conditioned for 3 months.

(3) Beans examined for mold at periods varying from 4 to 9 days.

As can be observed in table 3, the moisture content of the beans affected the physical appearance and aroma of the beans in each of the different curing treatments but did not produce any significant effect in the phenol value.

In all treatments, beans with moisture contents varying from 50 to 54 percent, had a fermented aroma generally lacking suavity and development. Beans containing 24 to 27 percent moisture had a more developed and suave aroma but little flexibility while beans with 31 to 34 percent moisture had a well developed and suave aroma and also possessed a high degree of flexibility.

Beans with lower moisture content were generally darker in color and as expected their vanillin crystallization was greater except in the sun-cured beans in which no crystals appeared. The beans killed by freezing were characterized by great flexibility even when their moisture content was low and had a reddish color.

No appreciable molding occurred in any of the treatments when the beans contained 24.2 to 26.5 percent moisture nor in the beans subjected to hot water or freezing having 30.7 to 50.5 percent moisture. Beans subjected to the ethylene and sun-killing procedures developed more mold than did those subjected to hot water or freezing. This may be due to the fact that in the former procedures the beans were not as thoroughly and uniformly killed. The effectiveness of the killing treatment is usually evidenced by a change in coloration which takes place in the beans during the sweating period or exposure to heat. The beans killed in hot water and by freezing became dark brown in color after a shorter period of sweating in electric ovens at 45°C., than the ethylene and sun-treated beans. Moreover, freezing and hot water treatment may have a sterilizing effect in the beans.

#### *Moisture Content of Foreign Beans*

Moisture contents were determined of several samples of beans from Mexico, Comores, Tahiti, Madagascar, and Guadeloupe, which are among the most important vanilla-producing countries of the world. Results are shown in table 4.

Table 4. Moisture content of cured vanilla beans from different countries

<i>Source</i>	<i>Moisture content</i>
	PERCENT
Mexico	40.3
Mexico (1)	38.3
Comores	35.4
Tahiti	37.5
Tahiti (1)	33.4
Madagascar	31.3
Guadeloupe (1)	17.8
Guadeloupe	15.2

(1) The moisture contents of these beans were determined several months after receipt

As can be seen in table 4, the moisture content was over 30 percent in the beans of all the countries except those of Guadeloupe.

#### SUMMARY

The moisture content of uncured vanilla beans was found to decrease with maturity. The average value obtained for whole beans entirely green was 81.2 percent and for whole, blossom-end yellow 79.2 percent. The moisture of split beans varied to a large degree with the extent and time of splitting.

Losses in weight in vanilla beans, other than those due to loss of moisture, were shown to be negligible during the curing process, regardless of the procedure used.

Although the greatest loss in weight in the beans occurred during the sweating and drying periods, a significant loss in weight also occurred during the first 3 months of the conditioning period. Moisture losses during conditioning were greater for beans of higher moisture content.

A nomograph is included showing the weights to which 100-pound lots of beans of different moisture contents should be reduced during curing to obtain a known moisture content in the end-product.

Moisture content was found to affect the appearance and aroma of the beans but did not produce any effect in the phenol value. Beans cured by different procedures with a moisture content of over 50 percent had a fermented aroma generally lacking suavity and development. Beans with 24 to 27 percent moisture had a more developed and suave aroma but little flexibility while those with 31 to 34 percent had well-developed and suave aroma and good flexibility. Beans with similar moisture content, sub-

jected to hot-water or freezing treatments were found to be less liable to mold infection than sun or ethylene-treated beans.

Commercial lots of vanilla beans from Mexico, Madagascar, Comores, and Tahiti were found to contain over 30 percent moisture.

Puerto Rican vanilla beans should be cured to a final moisture content of 30 to 35 percent if good flexibility and development and suavity of aroma, which are the principal factors affecting the sales value are to be obtained, judging from the results of our experiments.

### *Acknowledgments*

Appreciation is expressed to "La Cooperativa de Cosecheros de Vanilla de Puerto Rico" and to the Puerto Rico Reconstruction Administration for the loan of vanilla beans used in these experiments. The authors are also indebted to Mario Tomassini for help in the curing and to Merriam Jones for the construction of the nomograph.



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# THE VALUE OF UTILIZING EXISTING SHADE IN THE GROWING OF VANILLA

By

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*Vanilla grew more rapidly on underbrushed land with existing shade than on cleared land with replanted shade.*

The customary practice in the early plantings of vanilla in Puerto Rico was to clear the land and plant trees, such as the dwarf bucare (*Erythrina berteroana* Urban), for shade and support. This resulted in a delay of 1 or 2 years before actual planting of the vanilla could be made. From observations and trial it was found that many varied trees could be used as supports for vanilla and that usually the trees already on the land and producing shade were entirely adequate. Under this method planting can be started immediately, and by utilizing existing facilities, costs can be much reduced.

In 1937, a parcel of land on the grounds of the Puerto Rico Experiment Station at Mayagüez was prepared by clearing all trees and underbrush from one-half of the area and only the underbrush from the remainder. Both portions were planted with support stakes of dwarf bucare. On October 25, 1939, when the bucare in the completely cleared portion had grown sufficiently to afford proper shade, 100 8-node vanilla cuttings of uniform size and vigor were planted in both areas.

*Root formation was superior on vines under existing shade.*

A record of the number of roots formed on the cuttings in each treatment was made every second month for 1 year, as shown in table 1.

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(1) In cooperation with the Government of Puerto Rico.

Table 1. Cumulative root formation found on vanilla cuttings at bimonthly intervals after planting at the base of support trees on cleared land and on underbrushed land on October 25, 1939.

<i>Treatment</i>	<i>Roots formed</i>					
	Dec. 26	Feb. 26	Apr. 26	June 26	Aug. 26	Oct. 26
Planted on cleared land -----	<i>Number</i> 247	<i>Number</i> 222 (1)	<i>Number</i> 154 (1)	<i>Number</i> 135 (1)	<i>Number</i> 127 (1)	<i>Number</i> 121 (1)
Planted on land with existing shade.	409	419	426	433	436	426 (1)

(1) Decrease in number of roots was due to decay of some roots previously formed.

It is evident that the vines planted under existing shade produced the greater number of roots throughout the experiment. The root formation of the vines planted under existing shade, was over thrice as great as that of the vines planted on cleared land, and the rotting of newly formed roots was considerably delayed. This decay can probably be accounted for by the quality of the shade provided by the existing trees as compared to the limited shade provided in the cleared area by the bucare support trees.

*Amount of seed-piece rotting was considerably less on vines under existing shade.*

The information gathered regarding seed-piece deterioration is summarized in table 2.

Table 2. Extent of seed-piece rotting.

<i>Treatment</i>	<i>Decayed seed-piece internodes</i>					
	Dec. 26	Feb. 26	Apr. 26	June 26	Aug. 26	Oct. 26
Planted on cleared land	<i>Percent</i> 5.0	<i>Percent</i> 13.7	<i>Percent</i> 38.0	<i>Percent</i> 53.4	<i>Percent</i> 59.1	<i>Percent</i> 61.0
Planted on land with existing shade	1.6	1.6	2.1	3.3	4.4	6.8

As can be observed for table 2, of the vanilla cuttings planted, 61.0 percent developed stem rot on cleared land, while under existing shade only 6.8 percent were so affected. The greatest amount of stem rotting occurred during the dry season; at this time of the year the bucare supports shed their leaves and consequently many of the vanilla cuttings were exposed to direct sunlight, which apparently favored stem rotting.

*Amount of vegetative growth was superior in vines under existing shade.*

Data on the amount of vegetative growth are shown in table 3.

Table 3. Vegetative growth of vanilla vines planted on cleared land and on land with existing shade, October 25, 1939.

Treatment	Average stem growth per cutting					
	Dec. 26	Feb. 26	Apr. 26	June 26	Aug. 26	Oct. 26
	Inches	Inches	Inches	Inches	Inches	Inches
Planted on cleared land	2.76	7.32	10.96	16.84	26.26	21.41
Planted on land with existing shade	3.55	4.96	7.35	16.77	32.51	53.42

During the early part of the experiment, the amount of vegetative growth per plant on vines planted on cleared land was somewhat greater than that on the vines under existing shade. Vegetative growth per plant by June, 8 months after planting, was about equal for both treatments. From August to October, which period composes part of the rainy season in this district, the plants grown under existing shade greatly surpassed the other planting. It is evident that the amount of vegetative growth per plant was over twice as great as that of the vines grown on cleared land.

The total amount of vegetative growth of the cuttings together with the root germination and rotting of the seed pieces per treatment is shown graphically in figure 1.

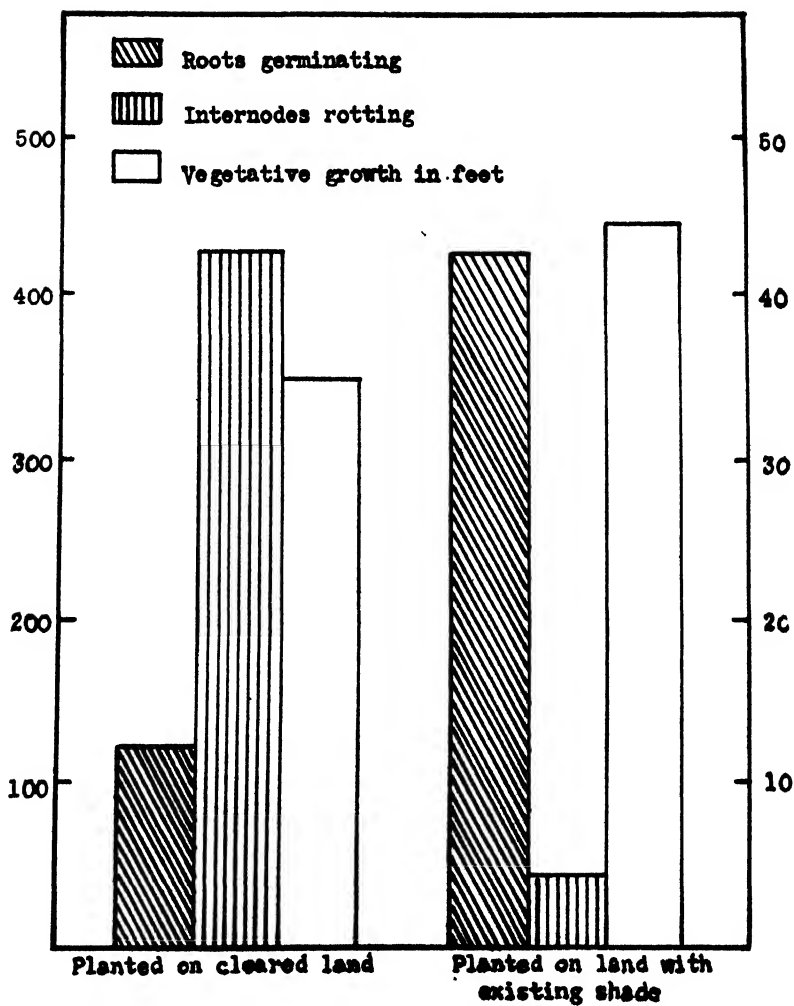


Figure. 1.

*Character of vine growth under existing shade was distinct from that on cleared land.*

From the beginning of the experiment it was observed that the internodes of the vines under existing shade were rather thin and long, as illustrated in figure 2, as compared to the short and thick internodes of the vines on cleared land, as shown in figure 3. The color of the former was dark green, characteristic of healthy normal plants, while that of the latter was an unhealthy yellow. This undesirable color of the vines on cleared land was doubtless due to excessive exposure to the sun's rays, intensified by the bucare supports shedding their leaves during the dry season.

This experiment clearly supports other observations that vanilla vines can be planted to advantage on land with existing shade with only a minimum amount of clearing. Furthermore, the planting can take place immediately after land preparation, eliminating a delay of from 1 to 2 years until the support trees have developed sufficient shade to protect the plants. Although bucare was used as a support in this experiment, it is now known that vanilla vines can be planted on available existing shade trees most of which serve the same purpose with equal satisfaction.



Figure 2. Character of vine growth of *Vanilla fragrans* vine planted on underbrushed land. Note the long, thin internodes. The leaves were long, narrow, and of a healthy dark-green color.



Figure 3. Character of growth of *Vanilla fragrans* planted on cleared land. Note the short, thick internodes. The leaves were short, thick, wide, cupped, and rather yellow due to excessive exposure to sun rays, intensified by the leaf shedding of the bucare supports during the dry season.



## SUMMARY

When vanilla was grown on bucare supports under existing shade it produced more root germination, less seed-piece rotting, and more vegetative growth than when grown on the same kind of support trees on cleared land. The differences between the two groups of vines were considerable. The vines under existing shade were of a dark-green color characteristic of healthy normal plants as compared to the yellowish color of the vines grown on cleared land. Because of the nature of dwarf bucare to shed its leaves during the dry season, at the time when the vanilla plant needs shade, it would be preferable to use this support, where needed, only under natural or existing shade.

*Acknowledgement*

The writer wishes to express his appreciation of valuable suggestions during the procedure of the experiment received from Dr. Arthur G. Kevorkian, former assistant plant pathologist and physiologist in charge of vanilla investigations.

# PRELIMINARY NOTE ON THE ADMINISTRATION OF NON-CONDITIONED PHENOTHIAZINE, IN SMALL DAILY DOSES, FOR THE CONTROL OF GASTROINTESTINAL PARASITES OF CATTLE IN PUERTO RICO <sup>1</sup>

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## INTRODUCTION

Shorb and Habermann (1940) reported that the presence of one per cent by weight of phenothiazine in the feces of sheep would prevent the development of nematode larvae and that .5 gram of this compound per day administered in the feed would inhibit the development of all nematode larvae in the feces of these animals except those of *Strongyloides papillosus*. Habermann and Shorb (1942) confirmed these findings in an experiment involving the voluntary ingestion of mixtures of phenothiazine and salt by infected sheep.

Cattle in Puerto Rico are commonly infested with a number of different species of gastrointestinal parasites. Since the scarcity of available clean pasture prevents the use of pasture rotation as a means of controlling these infestations, it was considered desirable, in view of the favorable results obtained by Shorb and Habermann (loc. cit.), to determine the effect of the frequent administration of small doses of phenothiazine over a long period of time on the cattle and on the parasites harbored by them.

## MATERIALS AND METHODS

Three herds of cattle on widely separated farms located on the north coastal plain of Puerto Rico were selected for these experiments. Herd 1

- (1) Published by permission of the Director of the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, P. R.
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was made up of 22 animals, 4½ to 24 months old, which normally grazed a relatively low-lying malojillo grass pasture, but were fed in addition chopped sugar cane trash or grass cut from a neighboring field, whichever was available at the time. Herd 2 was composed of 12 animals, 8 to 28 months old, which were kept in a feed lot containing some vegetation, but were fed chopped grass cut from a field used only for furnishing this type of feed. Herd 3 consisted of 15 animals, 7 to 11 months old, which were fed chopped grass, sugar cane trash, and concentrates. Some of the animals in the latter group were allowed to graze low-lying malojillo grass pasture.

The number of parasite eggs per gram of feces was first ascertained for each animal and the different species of parasites harbored by the animals was ascertained by noting the kinds of worm eggs present in the feces. Herds 1 and 2 were each divided into two equal groups in such a way that each group was passing approximately the same number of parasite eggs per gram of feces. Group 1 and Group 2 of Herd 1 were separated by a fence dividing the pasture originally occupied by the whole herd. Group 1 received ½ gram of nonconditioned phenothiazine per day per 100 pounds of live weight for 105 days. The compound was given in # 00 white gelatine capsules administered by means of a balling gun. Group 2 remained untreated as a control. At the end of the 105-day period, the treatment of Group 1 was discontinued and the treatment of Group 2 was begun at the same dose rate. The experiment was then continued for 69 days and was terminated because of the flooding of the pasture.

The two groups of Herd 2 were not separated from each other. Group 1 of this herd received phenothiazine in white gelatine capsules at the same dose rate as the animals belonging to Herd 1, but the compound was administered at weekly intervals. Group 2 of Herd 2 remained untreated as a control. Treatment was discontinued entirely at the end of 77 days, although the number of eggs per gram of feces was ascertained in both groups for an additional 89-day period.

Herd 3 was not separated into two groups. All of the animals in this herd received daily doses of phenothiazine mixed in the ground concentrate portion of their daily ration. These doses were equivalent to those received by the animals in Herd 1. Data on this herd were recorded for 175 days.

The animals belonging to Herd 1 were weighed at the beginning and at the end of the experiment. A weighing tape was used to estimate the weights of the animals at approximately weekly intervals during the

experiment. The same tape was used in estimating the weights of the animals in Herd 2. The animals in Herd 3 were weighed on a scale at monthly intervals.

Determinations of the number of parasite eggs per gram of feces were made on all animals at the intervals indicated in Figure 1, and the data recorded. No autopsies were made.

#### DATA OBTAINED

Nine animals from Herd 1, six from Herd 2, and nine from Herd 3 were very lightly infested at the beginning of the experiment, and passed so few worm eggs that the data were not included in this paper. Observations on one other calf were also omitted because the findings were not comparable to those obtained on the remaining animals. Data from 24 animals are recorded in Figure 1.

The average number of parasite eggs per gram of feces for six animals of each group belonging to Herd 1 are given in Graph A of Figure 1. These data show that the number of parasite eggs in the feces of the treated calves dropped markedly within one week from the beginning of treatment, while that of the control calves remained at a relatively high level for approximately two months. The drop in the egg count of the untreated calves which then occurred was probably due to the feeding of chopped sugar cane trash which may have decreased the rate of reinfection. When phenothiazine was administered to the previously untreated Group 2, the number of parasite eggs per gram of feces dropped to a very low level for the remainder of the experiment. The effect of discontinuing the treatment of Group 1 was not noticeable until about two months had passed when the number of worm eggs per gram of feces increased slightly. The number of parasite eggs passed by the animals in Group 2 which were treated in the last part of the experiment did not increase.

The average number of parasite eggs per gram of feces in three animals in each of the two groups belonging to Herd 2 is shown in Graph B of Figure 1. Although the number of eggs in the feces of the untreated cattle showed marked fluctuations, the same result as that obtained in the previous experiment following treatment is shown in this graph. The discontinuance of the treatment of Group 1 did not bring about an increase in the number of parasite eggs in the feces of these animals.

The average number of parasite eggs per gram of feces in six animals of Herd 3 is shown in Graph C of Figure 1. These data also corroborate the results of the experiment shown in the preceding graphs.

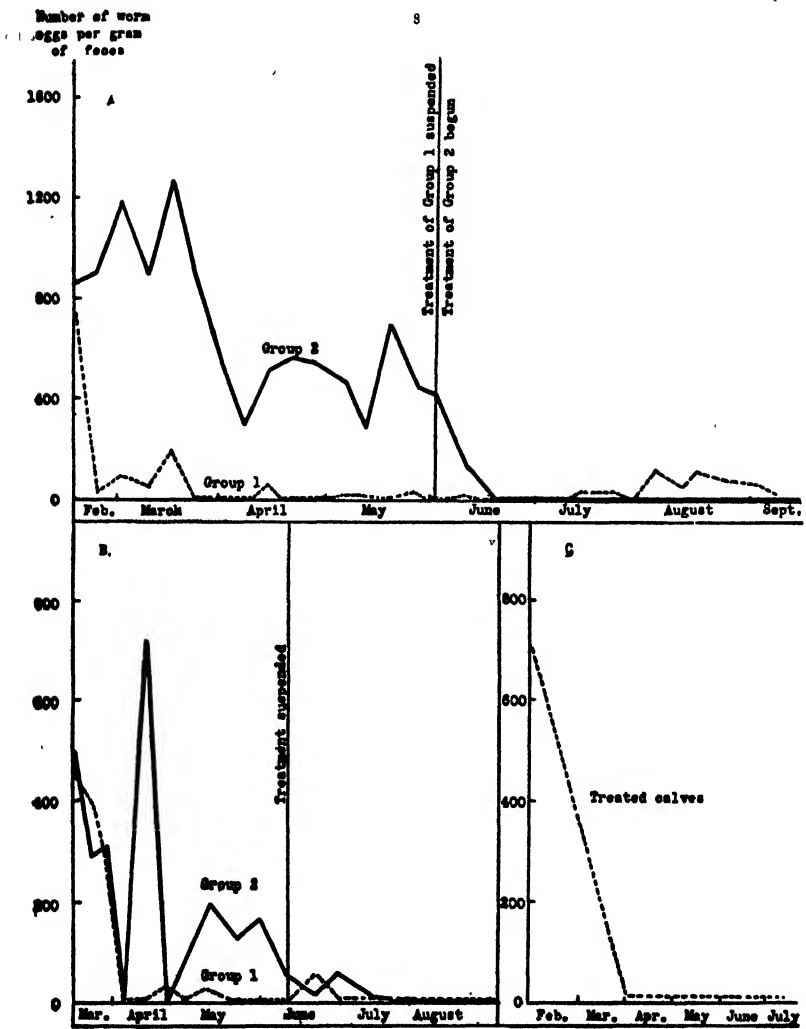


Figure 1. Average number of worm eggs per gram of feces passed by treated and untreated calves.

The data obtained from these three experiments show that the daily or weekly administration of nonconditioned phenothiazine to cattle infected with gastrointestinal parasites at the dose rate of  $\frac{1}{2}$  gram per 100 pounds of live weight per day decreased the number of parasite eggs passed unto the pasture by the infected animals to a negligible number.

While a general reduction in the number of parasite eggs in the feces occurred following treatment with phenothiazine, the reduction was not evenly distributed among the various species of parasites harbored by the infected animals. The eggs of the stomach worms, *Haemonchus contortus* and *H. similis*, the intestinal hair-worms, *Trichostrongylus* spp., the hook-worm, *Bunostomum phlebotomum*, and the nodular worm, *Oesophagostomum radiatum*, disappeared relatively soon after the beginning of treatment. Those of the small intestinal worms belonging to the genus, *Cooperia*, the thread worm, *Strongyloides papillosus*, and the broad tapeworms, *Moniezia* spp. were not noticeably reduced in number by the treatment.

#### *Effect of daily doses of phenothiazine on cattle and cost of treatment*

The only visible sign that the animals were being treated with phenothiazine was the reddening of the urine upon exposure to air. So far as could be observed clinically and from the data obtained on the weight gained by the treated and untreated cattle, no ill effects could be detected during the entire experiment.

At the time these experiments were carried out, nonconditioned phenothiazine was selling at approximately \$1.50 per pound. At this price, the daily expense of treating the animals in the three herds varied from .58 to .81 cents per head, the large figure representing the cost of treating the animals in Herd 3 where a slight excess of the compound was mixed with the feed in order to allow for losses due to the unavoidable scattering of the phenothiazine-feed mixture.

## DISCUSSION AND SUMMARY

The results of the foregoing experiments show that daily doses of  $\frac{1}{2}$  gram of nonconditioned phenothiazine per 100 pounds of live weight administered to cattle infected with gastrointestinal parasites reduced the number of worm eggs passed unto the pasture by the infected animals. The eggs of the stomach worms, *Haemonchus contortus* and *H. similis*, the small intestinal hair-worms, *Trichostrongylus* spp., the hookworm, *Bunostomum phlebotomum*, and the nodular worm, *Oesophagostomum radiatum*, disappear relatively quickly from the feces of the treated animals. The eggs of *Cooperia* spp., *Strongyloides papillosus*, and the broad tapeworms, *Moniezia* spp., were not markedly reduced in number by this treatment. These results confirm those of Porter, Simms, and Cauthen (1941) showing that phenothiazine has little effect on the last three parasites mentioned. The dose rate used in these experiments compares favorably with that suggested by Shorb and Habermann (loc. cit.) for the prevention of the development of nematode larvae in the feces of sheep.

*Acknowledgements*

The writers are deeply grateful for the very helpful cooperation of Mr. Sergio Dávila, Mr. W. H. Wildman, and Dr. Morales Otero, who permitted the use of their cattle for these experiments.

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**A NEW SPECIES OF PHLOEONEMUS FROM PUERTO RICO  
(COLEOPTERA: COLYDIIDAE)**

**By**

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In a small collection of Coleoptera received for identification from L. E. Martorell the following new species was found.

**PHLOEONEMUS MARTORELLI, new species**

Oblong-elongate, rather strongly flattened, not distinctly pubescent, dark reddish brown, the antennae and palpi slightly paler.

Head broad, flat, sides dilated, the dilation extending backward to middle of eyes, broadly, vaguely depressed on each side toward eyes, obtusely carinate above eyes, the carina extending from posterior fourth of eye to a short distance in front of eye; surface very finely, densely granulose, finely, shallowly punctate anteriorly, finely, densely, irregularly reticulate posteriorly.

Pronotum strongly transverse, much wider than head, widest near posterior angles; sides slightly rounded, nearly parallel along basal half, vaguely converging anteriorly, the margins finely crenulate; apical margin deeply emarginate, broadly rounded and thickened at middle; apical angles projecting and rather acute; base slightly arcuate at middle, vaguely sinuate on each side; posterior angles obtusely rounded; disk moderately convex, broadly flattened toward lateral margins, with three obtusely rounded, longitudinal costae on each side of middle, median one extending from base to apex, abruptly bulging externally at middle and connected posteriorly to the second costa, which extends backward to base, and the third costa vaguely sinuate, extending from base to apex; surface rather coarsely, densely granulose, the granules very irregular in shape, and contiguous at some places.



Elytra subequal in width to pronotum; sides parallel to apical fourth, then arcuately converging to tips, which are conjointly broadly rounded, the lateral margins acute and vaguely crenulate. Each elytron with five sharply defined, longitudinal costae; sutural and second costae extending from base to apex; third and fourth costae extending from base to near apex; fifth costa extending backward from humerus, and connected to lateral margin near apex; intervals between costae (except lateral one) with two rows of rather coarse punctures, separated from one another by about their own diameter, and the interval between punctures in each row with a narrow, longitudinal carina extending from base of one puncture to apex of following puncture, the interval between rows of punctures nearly smooth.

Abdomen beneath more or less irregularly imbricate-puncture; prothorax coarsely, transversely rugose.

Length 4.7-6 mm., width 2-2.7 mm.

*Type locality.* — Guánica. Puerto Rico.

*Type and paratypes.* — In the United States National Museum, No. 56541. Paratypes in the collection of L. F. Martorell.

Described from ten specimens (one type) all collected at the type locality, January 31, 1940, on the gummy exudations on the trunks of *Zanthoxylon flavum* trees, by L. F. Martorell.

This species is closely allied to *Phloeonemus haroldi* Reitter, described from Cuba, but it differs from that species in being larger, in having the longitudinal costae on the elytra not interrupted, the carinae above the eyes obtusely rounded on the tops, and the costae on the pronotum more distinct with the lateral one on each side vaguely sinuate.

# A NEW AMBLYCERUS AFFECTING SEEDS OF PROSOPIS CHILENSIS IN PUERTO RICO AND HISPANIOLA

By

JOHN COLBURN BRIDWELL,

United States National Museum  
Washington, D. C.

Among the insects found affecting the seeds of forest trees in Puerto Rico by L. F. Martorell is a new species of the bruchid genus *Amblycerus* here described and named in his honor. The same species has been intercepted in material originating in Haiti and the Dominican Republic by inspectors of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

## AMBLYCERUS MARTORELLI Bridwell, new species

Nearly the size and habitus of *Amblycerus robiniae* (Fabricius) (= *Spermophagus haffmanseggi* of the Leng Catalogue not of Gyllenhal) but lacks the black integumentary areas of that species, is smaller, and has differently shaped pronotum and scutellum, shorter calcaria and numerous differences in sculpture. Reddish brown with appressed pubescence, uniformly yellowish cinereous above, and pale beneath, nearly evenly disposed and partly concealing the surface sculpture, without blackish hairs except for single black hairs in the larger punctures of pronotum and elytral intervals. Pectus often infusate, sternites with ill-defined paler margins.

Length, 5-6.5; width, 3-3.5 mm.

Eyes emarginate for about one-fourth their length, coarsely faceted, strongly convex, projecting about one-half their width; front at clypeus separating the eyes by about one-half their width, strongly punctulate, without coarser punctures, with only a slight vestige of a glabrous unpunctured line near clypeous, mentum without punctures. Antennae with 3 narrow joints at base, joints 2 and 3 together about as long as joint 1 and longer than 4, joints 4-10 longer than broad, compressed and expanded with inner apical angles produced, these joints subserrate and closely applied to each other.

Prothorax about as broad at base as the elytra, transverse, dorsum coarsely and rather densely punctured on the sides, a broad longitudinal median area without these punctures, impressed lines along lateral margins above and below ending far from the anterior margin, flanks without coarse punctures; prosternum very narrow between the coxae, extending slightly beyond them, slightly expanded and truncate at apex, not received in any special structure of mesosternum; this nearly vertical, flat, hairy, and truncate at apex, meeting metasternum at an obtuse angle; metasternum not gibbous, with apex set off by the impressed marginal line; scutellum parallel sided, oblong-subquadrate, pointed at apex, emarginate on either side of the point, the lateral angles rounded.

Elytra about thrice as long as prothorax, widest near middle, broadly, obliquely, subtruncately separately rounded at apex, intervals 2, 4, 6, and 8 slightly costate giving a slight vittate effect, intervals dotted with fine darkish punctures each bearing a single black decumbent hair. Pygidium nearly plane, oblique, about as broad as long, margins converging in a convex curve to the broadly truncate or rounded apex, disc infusate, margins pale, a pale pubescent longitudinal line, punctured except for a small subbasal area on either side. Last sternite longer than the preceding in female, shorter than preceding in male.

Hind coxa with about 30 irregularly disposed, rather coarse shallow punctures on the large pubescent area and with several fine strongly impressed punctures on the glabrous shining area near the insertion of the trochanter. Inner and outer carinae of ventral margin of hind femur obsolescent on basal half, inner carina unarmed as is usual in *Amblycerus*. Calcaria of hind tibia but little unequal, as 5 to 4, longer outer calcar not half as long as basal tarsal joint; outer dorsal surface of hind tibia with a line of closely placed punctures extending from base to apex, ventral surface with two lines of punctures where it meets the outer and inner faces in an even curve, apex obliquely truncate with about five rounded teeth at dorsal apex.

Described from 41 specimens in the United States National Museum, reared from pods of *Prosopis* (or *Neltuma*) *chilensis*, labeled as follows:

Type a male, also 16 female and 8 male paratypes: from seed-pods of *Neltuma juliflora* (= *chilensis*), P. R. Acc. No. 785-40, Guánica, PUERTO RICO, 12-5-40, L. F. Martorell.

Five female and 3 male paratypes, from mesquite, HAITI, 27-9-35, N. Y. 47420.

Six female and 2 male paratypes, from *Prosopis chilensis*, DOMINICAN REPUBLIC, Chicago No. 1126, 21-xi-41. These were submitted by the Division of Plant Quarantine, Bureau of Entomology and Plant Quarantine. U. S. N. M. Type No. 56542.

The transverse pronotum, coarsely punctured laterally and finely punctulate on a broad median area; the impunctate mentum and flanks of pronotum; the very narrow prosternal process slightly wider at apex; the hind tibia with dorso-lateral line of punctures and with subequal calcaria less than half as long as basal tarsal joint; these characters separate *A. martorelli* from *A. robiniae*, *A. hoffmanseggi*, and most species of *Amblycerus* known to me. *A. hoffmanseggi* (Gyllenhal), *A. nigromarginatus* (Motschulsky), *A. obscurus* (Sharp), and *A. baeri* (Pic) may be one species judging from the descriptions, and the species I have doubtfully determined as *A. hoffmanseggi* resembles *A. martorelli* closely but the calcaria are more unequal in length, the longer outer one being one and one-half times as long as the inner and more than half as long as the basal tarsal joint and the mentum is distinctly punctured. It is found from Mexico to Brazil breeding in the seeds of several species of *Cassia* (sens. lat.). From *A. piurae* (Pierce), which breeds in *Prosopis* in Peru, *A. martorelli* differs in coloration, no part of its body and appendages being as dark as the nearly black antennae, legs, disc of elytra, breast, and sternites of *A. piurae*. They are not mere variants since the median lobe of the aedeagus in *martorelli* is much more expanded near apex and more nearly parallel sided in *piurae*. Typical examples of *A. testaceus* (Pic) from the Chaco of Argentina are very unlike *martorelli*, but I have seen associated with these entirely testaceous individuals others resembling *martorelli*. These have, however, only a few rather fine punctures on the sides of the pronotum, only visible when the pubescence is removed. Its host plant is still unknown, but seems to be neither *Prosopis* nor *Cassia*.

*Amblycerus martorelli* has been compared with all the West Indian species of *Amblycerus* in the United States National Museum, including nearly all the described species and numerous undescribed forms, and it resembles none of them at all closely. Its presence in Puerto Rico and Hispaniola seems certainly the result of accidental introduction from some unknown region of North or South America where it lives at the expense of *Prosopis chilensis*. This plant occurs in the West Indies only where it has been introduced. For the most part such introductions have occurred in recent years, but in Jamaica before the middle of the eighteenth century.



## A NEW ENCYRTID PARASITIC IN THE EGGS OF HESPERIIDAE

By

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Specimens of an encyrtid reared from eggs of *Prenes* sp. in Puerto Rico, recently submitted for identification, appear to represent a new species.

### *Ooencyrtus prenidis*, new species

This new species is apparently most closely related to *Ooencyrtus latiscapus* Gahan but may be distinguished at once by the much narrower antennal scape of the female and by the wholly yellowish-testaceous legs. The color of the legs is similar to that of the legs of *submetallicus* (Howard) but the antennae are much shorter and more strongly clavate and the sculpture on frons and scutellum is shallower and less distinct.

*Female*.—Length 0.75 mm. Head as broad as thorax, rather deeply concave behind; fronto-vertex moderately narrow, the distance from upper end of scrobal depression to posterior margins of vertex equal to about one and one-half times the shortest distance between the eyes; frons and vertex with distinct but shallow reticulate-punctate sculpture; face and cheeks indistinctly sculptured, nearly smooth; ocelli in a slightly obtuse triangle; scrobal cavity triangular, nearly acute dorsally; mandibles with three very short, blunt teeth; eyes with very sparse, inconspicuous pile. Antenna relatively short, strongly clavate; scape slightly thickened medially, approximately four times as long as broad; pedicel nearly twice as long as broad and a little less than one-third the length of scape; funicle gradually thickening from base toward apex, each segment with an irregular whorl of hairs which are a little longer than the segments from which they originate; first funicular segment narrower than the pedicel and a little longer than broad; remaining segments about as long as broad; club indistinctly 3-segmented, nearly twice as broad as sixth funicular segment, elongate

ovate in outline, approximately as long as the four preceding funicular segments combined and clothed throughout with much shorter and finer hair than on the funicle.

Thorax only a little longer than broad, convex dorsally; pronotum very short, mostly concealed from above; mesoscutum much broader than long, weakly reticulated, and moderately clothed with short, brownish hairs; scutellum moderately convex, about as long as mesoscutum and with similar sculpture on basal two-thirds, the apical third smooth and shining, a pair of rather long, erect bristles at extreme apex and a few shorter hairs scattered over the basal sculptured portion; propodeum very short, smooth and shining; mesopleuron nearly smooth. Legs normal. Forewing a little less than two and a half times as long as broad, extending far beyond apex of abdomen: marginal vein slightly longer than broad and not quite so long as stigmal vein; postmarginal vein about as long as marginal, indistinct; discal cilia basad of the hairless streak distinctly sparser and coarser than those distad of it.

Abdomen much broader than long, subtriangular, as broad as thorax but much shorter, its surface without distinct sculpture; ovipositor not exerted.

Head and dorsum of thorax black with a distinct metallic luster, the mesoscutum usually with a slight greenish tinge, and the scutellum bronzy basally but with the smooth apical portion distinctly greenish; under side of thorax dark brownish; legs, including all coxae, yellowish testaceous; wing hyaline; tegulae concolorous with mesoscutum; abdomen brownish black. Antennal pedicel and segments 3-6 of the funicle more or less dark brownish; scape, first and second funicular segments, and club pale testaceous.

*Male*.—Length 0.75 mm. Less robust than the female: fronto-vertex broader than long; ocelli in a distinctly obtuse triangle; ocellular line approximately equal to half the diameter of a lateral ocellus; scrobal cavity rounded dorsally. Antenna long, not clavate; scape not thickened; pedicel about as broad as long; funicular segments all of about the same width as pedicel but longer, subequal to one another or increasing very slightly in length from first to last, the first segment about one and a half times as long as broad, the sixth sometimes a little more than twice as long as broad, each segment clothed with hairs which are somewhat longer than the segment bearing them; club about as long as two preceding segments combined and scarcely broader than funicle. Abdomen about as long as broad, rather sharply triangular. Antenna nearly uniformly pale yellowish,

the pedicel and base of scape more or less fuscous; head, thorax, and abdomen black dorsally with a distinct coppery tinge; underside of thorax brownish black; legs yellowish testaceous, the coxae, hind femora, and basal half of hind tibiae infuscated with brownish. Otherwise like the female.

*Type localities*.—Salinas and Quebradillas, Puerto Rico.

*Type*. — U. S. National Museum No. 56540.

Described from 18 females (1 holotype) and 8 males (1 allotype) reared from eggs of *Prenes* sp. on sugarcane at Salinas, Puerto Rico, by L. F. Martorell in June 1942; also 2 females and 2 males reared at Quebradillas, P. R., Oct. 20, 1938, from *Prenes* sp. eggs.





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## STUDIES ON TOMATO MOSAIC IN PUERTO RICO

### A NEW MOSAIC DISEASE OF TOMATO

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#### INTRODUCTION

Tomatoes are extensively grown in Puerto Rico for home consumption and for the export trade. A fair idea of their importance is shown by the fact that 1500 acres of tomatoes were planted in the vicinity of Villalba and Jayuya during the four-month crop of 1941-42.

Tomato mosaic is a serious and widespread disease and is responsible, in many cases, for great losses. The nature of the causal element or elements of tomato mosaic in Puerto Rico has not been ascertained so far. A survey and study of the virus or viruses causing tomato mosaic; conditions under which it becomes serious, methods of transmission, host range, and the reaction of different commercial and non-commercial varieties of tomatoes which are mosaic resistant or tolerant to the virus or viruses encountered, are necessary before attempting to produce commercial varieties of tomatoes adapted to local conditions.

During the summer and fall of 1942, the Agronomy Department of the Agricultural Experiment Station of the University of Puerto Rico was confronted with a mosaic-like disease which rendered useless the tomato seedlings propagated for distribution among farmers. Some of these seedlings planted in the Station propagating garden were severely affected with the disease. The tomato plants found affected were of the varieties Marglobe, King, and Newark.

#### SYMPTOMS

Under field conditions, during the hot and humid summer and fall months of 1942, the affected tomato plants showed, in their late stages of growth, a characteristic faint yellowish mottling of the leaves with little or no leaf

distortion. Often the mottling was barely perceptible, or it might altogether disappear. Such affected tomato plants apparently grew normal and produced fairly good crops.

Tomato plants found affected early in their development, showed a pronounced retardation in upward growth, with a condensation of the axis, a progressive decrease in leaf size and various forms of leaf deformation. Necrosis of the growing tips developed frequently. Necrosis also occurred in leaves showing marked reduction in size and deformation. The midribs and lateral veins of affected leaves showed, especially on the underside, a peculiar purplish coloration followed by necrosis, the latter extending throughout the leaf forming large, irregular blotches. Eventually when the whole lamina was affected and had disintegrated, the midrib was left bare. The stems of such severely affected plants became heavily streaked with broad, black and short, or narrow, longitudinal streaks of varying lengths. New shoots were produced below the affected parts of the axis and also frequently became equally affected. On new shoots, formation of leaves was almost always reduced to malformed, purplish outgrowths which finally dried up. The flowers of such severely affected plants were commonly malformed and abortive and, if any fruits were produced, they were small and streaked.

Under greenhouse conditions, young, artificially inoculated tomato plants showed the mottling symptoms described above with some slight inward curling of the margins and tips of the leaves. Other tomato plants showed tip blight, bushy growth, and some of them succumbed to infection. Under such conditions young tomato plants produced small, narrow or filiform leathery leaves, with a peculiar bronzing previous to blighting.

#### MATERIALS AND METHODS

Tomato leaves (1) with mottle symptoms, (2) without mottle symptoms and (3) necrotic leave tissue, free from extraneous material, were taken from naturally infected tomato plants and macerated respectively in sterilized mortars. The individual macerates were immediately expressed through sterile cheesecloth. Each inoculum was prepared by diluting the filtrated juices in nine parts of distilled, sterile water, and used immediately. These macerates will be referred to as extracts (1), (2) and (3) in the course of this paper.

The virus once isolated and identified was kept in pure culture in tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and pepper (*Capsicum frutescens* var. California Wonder). The extracts prepared with leaves of the infected plants were identified by the name of the source plant, i.e., tomato extract, tobacco extract and pepper extract.

All inoculations were performed by rubbing gently the upper side of the leaves of the differential host plants with Mikado No. 5 brushes with bristles cut to  $\frac{1}{2}$  inch, dipped, in every instance, in the respective inoculum. The inoculated plants were kept in an insect-free greenhouse. Temperature inside the green house fluctuated from 70°F. during the night to 90°F. at daytime. Relative humidity fluctuated from 30 to 80%.

Differential and tomato host plants used throughout the work were grown in sterilized compost soil in 5-inch pots in insect-free insectaries inside greenhouses. Inoculations were performed when the young plants had developed 3 or 4 pairs of leaves. Inoculated plants were kept growing until flowering to observe their reaction throughout their whole life-span.

#### EXPERIMENTAL RESULTS

##### *The Causal Agent*

Young plants of tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and *N. glutinosa* were respectively inoculated with each of the three virus extracts.

Tomato plants reacted to inoculation with extracts (1) and (2) with systemic mottling and inward curling of the margins and tips of the leaves. The same extracts produced systemic vein clearing, vein banding, and a chlorotic mottling in tobacco. *N. glutinosa* reacted with vein clearing, systemic mottling and chlorosis.

Percent of infection obtained by inoculating with extracts (1) and (2) was very low. None of the host plants utilized reacted to inoculation with extract (3) (Table I).

Absence of local necrotic spots on *N. glutinosa* when inoculated with extracts (1) and (2) discarded the possibility that the mosaic of tomato might be due to *Tobacco Virus 1*, Johnson.

Young cucumber (*Cucumis sativus*) and black-seeded cowpea (*Vigna sinensis*) plants failed to react when inoculated with any of the virus extracts.

In order to determine the possible relation of the virus or virus entities attacking tomatoes to that recently reported in peppers by Roque and Aduar (6), pepper plants (*Capsicum frutescens* var. Large Bell Hot) were respectively inoculated with tomato, tobacco and *N. glutinosa* virus extracts. Within five to six days the inoculated pepper plants showed the characteristic symptoms described on this host when infected with the pepper virus as reported by these workers, i.e. systemic vein necrosis, stem streak, and finally defoliation and death. *C. frutescens* var. California Wonder, reacted with vein clearing, mottling, and stunted growth.

The physical properties of the tomato virus under study in relation to

mechanical and insect transmission, longevity in vitro, thermal inactivation, and dilution end point, were identical to those already reported for the pepper virus found by Roque and Adsuar (6), demonstrating that the virus found in tomato is identical to their pepper virus.

TABLE I

*Reaction of Host Plants to Virus Extracts from Various Parts of Affected Tomato Plants. Plants Inoculated in August 1942 and Kept at All Times Inside the Greenhouse in Insect-Free, Screened Rooms*

Host Plant	Virus Source	No. Inoculated	Dis-eased	First Sympt-oms days	Reaction
Tomato v. Mar-globe	Mottled leaves	20	7	15-20	Faint mottled leaves
	Un-mottled leaves	20	5	15-20	Faint mottled leaves
	Necrotic leaf tissue	20	0	0	Negative
Tobacco v. Virginia	Mottled leaves	20	5	7-8	Vein clearing and faint mottle
	Un-mottled leaves	20	9	7-8	Vein clearing and faint mottle
	Necrotic leaf tissue	20	0	0	Negative
<i>Nicotiana glutinosa</i>	Mottled leaves	20	4	25-30	Faint mottle leaves
	Un-mottled leaves	20	2	25-30	Faint mottle leaves
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. Large Bell Hot	Mottled leaves	20	11	5-6	Vein clearing and necrosis, defoliation and death
	Un-mottled leaves	20	9	5-6	Vein clearing and necrosis, defoliation and death
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. California Wonder	Mottled leaves	20	4	10-12	Systemic leaf chlorosis and deformation
	Un-mottled leaves	20	6	10-12	Systemic leaf chlorosis and deformation
	Necrotic leaf tissue	20	0	0	Negative

#### *Virus Source and Virulence*

Infection of tomato and tobacco with virus extracts from affected tomato plants is not so readily obtained as when the virus is taken from *N. tabacum* var. Virginia or from *N. glutinosa*. An increase in virulence seems to result when the virus is passed through these species of tobacco. De-

creased virulence was noticed when the virus was obtained from tomatoes (Table II).

While infection of *N. glutinosa* by inoculation with tomato mottle-leaf extracts is difficult, however, a high percentage of infection was obtained by inoculating *N. glutinosa* with extracts of either affected *N. tabacum* or *N. glutinosa* plants.

The symptoms in *N. tabacum* are more obvious and more severe. Generally *N. tabacum* var. Virginia, showed vein clearing, faint mottling and vein banding without deformation. When inoculated with a virulent tobacco-virus extract the leaves showed deformation and peculiar dark

TABLE II

*Virus Source and Virulence. Young Host Plants Kept Inside Insect-Free Greenhouse*

Host	Virus Source	Plants Inoculated	Plants Diseased	Symptoms	Reaction
Tobacco v. Virginia	Tomato	20	8	7-8 days	Vein clearing, mottle
	Tobacco v. Virginia	20	17	5-7	Vein clearing, mottle
	<i>N. glutinosa</i>	20	15	5-7	Vein clearing, mottle
<i>N. glutinosa</i>	Tomato	20	0	0	Negative
	Tobacco v. Virginia	20	13	7-8	Faint mottle, chlorosis and death
	<i>N. glutinosa</i>	20	15	7-8	Faint mottle, chlorosis and death
Pepper v. Large Bell Hot	Tomato	20	7	5-6	Necrosis, death
	Tobacco v. Virginia	20	20	5-6	Necrosis, death
	<i>N. glutinosa</i>	20	20	5-6	Necrosis, death
Cucumber	Tobacco v. Virginia	20	0	0	Negative
	<i>N. glutinosa</i>	20	0	0	Negative
		20	0	0	

green markings. *N. glutinosa* inoculated with virulent tobacco-virus extracts reacted within five to six days in contrast with twenty or more days when inoculated with attenuated tomato virus extracts, a high percentage of plants becoming infected. Young *N. glutinosa* infected with the virulent virus showed marked systemic chlorosis, blistering and leaf mottling and finally wilting. This wilting reaction is not produced when inoculation is performed with apparently attenuated virus extracts from tomato.

Tomato plants inoculated with tobacco and *N. glutinosa* virus extracts, responded readily with characteristic leaf mottling, puckering and some-

times with leaf deformation and tip blight. The virus entity on tomatoes inoculated with these two virus extracts is consistently recovered by inoculating expressed sap of infected tomatoes on tobacco and *N. glutinosa*. When a more sensitive test is to be performed there is no better indicator than young, actively growing Large Bell Hot pepper plants. This variety of pepper is so sensitive that it has shown the presence of the virus entity in infected old pepper plants, and in *N. tabacum* v. Virginia and *N. glutinosa* which had been previously inoculated with attenuated virus extracts from tomato and old pepper plants. These tobacco plants had shown a mild expression of the disease becoming later symptomless carriers.

#### DISCUSSION AND CONCLUSIONS

The present studies demonstrate the presence of the pepper virus (Roque and Adsuar) (6) in tomato plants affected with mosaic. The virus causes a characteristic faint, yellowish mottling and downward puckering of tomato leaves. This has been repeatedly verified by inoculating tomato plants with (1) pepper virus obtained from tomato mosaic affected plants, (2) tomato mosaic virus passed through tobacco and *N. glutinosa* and (3) with pure cultures of the pepper virus thru commercial peppers. The identity of the virus has been substantiated in all cases by the severe vein-necrosis reaction it produces in the Large Bell Hot pepper variety.

Necrotic symptoms, in the form of tip blight and streak, were found to be sometimes associated with the faint mottling, yellowing and puckering so characteristic of the pepper virus in tomato. In that respect it can be stated that it has not been possible to reproduce consistently tip blight necrosis when healthy tomato plants were inoculated either with pepper virus extracts obtained directly from tomato or passed through tobacco. Faint mottling, yellowing and puckering were invariably obtained when such inoculations were performed. In a few cases tip blight symptoms appeared in tomato plants inoculated with virus extracts from tomatoes showing tip blight. This reaction was lost in subsequent transfers, only faint mottling, yellowing and puckering of leaves being observed.

It seems likely therefore, that the blight and necrosis sometimes found associated with tomato mosaic is due, either to a single entity, or to its interaction with the pepper mosaic virus in the tomato plant. In any case the failure to artificially reproduce consistently the tip blight symptoms in plants grown in greenhouses might be attributed, among other causes either to ignorance as to its method of transmission or to a very rapid inactivation of the responsible agent "in vitro."

The fact remains however, that tomato tip-blight like viruses very similar to the one described here have been encountered and reported by other investigators. Among those so far described, that responsible for the

Oregon tip blight of tomatoes (4-5) is very similar, at least as to clinical syndrom. Until more is known about the tip blight virus found in Puerto Rico in respect to transmission and physical properties, its nature will remain a mooted question.

The apparent loss of virulence resulting when the tomato mosaic virus is consecutively passed through tomato or submitted to a long sojourn in either tomato or old pepper plants, as well as its increased infectivity when transferred to tobacco, are phenomena already well established for other viruses. Its importance in connection with this or similar work is that it serves to explain the low percentages of infection, or even at times, the failure to transmit the disease when the virus is passed through different hosts.

Failure to recover the virus entity from dried leaves of affected tobacco plants and from necrotic tomato leaves suggest the possibility of control by cutting affected plants and letting them dry "in situ." As the virus is transmitted mechanically, care should be taken not to carry it from affected tomato, tobacco or pepper plants while working in seed beds. Pepper is extensively grown in Puerto Rico and is an important virus source.

#### RESUMEN EN ESPAÑOL

Se confirma la existencia en Puerto Rico de un mosaico del tomate causado por el virus del pimiento tal como ha sido informado por Roque y Adsuar (6).

La enfermedad se caracteriza por un matizado del follaje acompañado frecuentemente por una necrosis de las hojas y tallos jóvenes. Esta suele extenderse en estrías negruzcas al largo de los tallos.

Se ha demostrado que el virus pierde su poder infeccioso en tejidos necróticos.

El hecho que los síntomas necróticos no acompañen necesaria y consistentemente al matizado de las hojas, hace pensar que haya mas de un virus envuelto en la compleja manifestación de la enfermedad.

Todo parece indicar que la necrosis que acompaña al matizado de las hojas se deba o bien a una o varias entidades distintas al virus del pimiento, o a la interacción de estas con el virus del pimiento.

Se ha observado una decidida atenuación del virus del pimiento al recuperarse del tomate. Por otro lado se ha comprobado que éste puede recobrar su virulencia al cultivarse en tabaco.

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## PLATE A

FIG. 1. Terminal shoot of naturally infected tomato plant showing symptoms of mosaic in the form of leaf deformation, a peculiar purplish coloration of growing tips followed by necrosis and broad, black, short or narrow, longitudinal streaks on stem and branches.



(García and Adsuar, Studies on Tomato Mosaic in Puerto Rico)



# ACROTHECIUM LEAF SPOT OF *BASELLA RUBRA* L.

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*Basella rubra* L. (*B. alba* L.), known commonly as Malabar Nightshade in Asia and "Espinaca del País" in Puerto Rico, is a succulent annual or biennial vine of Asiatic origin. It is grown in Puerto Rico and in other regions of the Caribbean area for its edible foliage composed of alternate, entire leaves.

At present, there is only a small acreage planted in Puerto Rico. Some few acres are grown to supply public school lunchrooms. Vines are sometimes seen growing on home gardens. This plant can supply a steady and cheap crop of leaves throughout the entire year. The succulent leaves can be used green or cooked, being more appetizing in the latter manner.

The small acreage of this crop in Puerto Rico is to be attributed to the lack of knowledge among farmers of the advantage of having a cheap source of greens for home consumption the year round. It is a well known fact that one of our great dietary problems in Puerto Rico is the insufficiency of roughage in our meals. Vegetable growing in Puerto Rico is a risky proposition and an expensive one for the average farmer. The small farmers could solve in part their roughage problem by a more liberal use of this fast-growing crop. A few plants suffice to take care of the family needs.

A review of the available literature reveals but very few organisms attacking *Basella rubra* L. From Brazil (9), three leaf parasites causing appreciable damage to this plant have been reported: A *Stagonospora*, a *Cercospora* and a *Phyllosticta* species.

In Puerto Rico the writer observed a severe leaf spotting of *B. rubra* among plants grown in a vegetable garden of the W.P.A. (Work Project Administration), located at the Experimental Station grounds, at Río Piedras, P. R. The serious outbreak occurred during the hot, humid summer of 1943.

The disease was characterized by numerous, reddish, subcircular leaf lesions and reddish, elongate vine spots. A parasitic fungus of the genus *Acrothecium* was almost invariably isolated from the red leaf and vine lesions. This organism has never been reported in Puerto Rico nor apparently elsewhere; hence research was conducted to elucidate the host-parasite relationship, under our environment, in the hope of finding a practical measure of control of the disease.

## THE DISEASE

The disease caused most serious damage to the foliage, impairing its food value. Primary infections of leaves appeared as a few small yellowish-

brown or reddish spots which enlarged slowly when weather conditions were relatively dry. Leaf spots sometimes showed yellowish, marginal zones, but the affected area remained sharply defined. When the enlargement of the leaf lesions was arrested, a distinctive reddish halo was formed around the innermost, necrotic dirty-brown leaf tissues of the lesions.

During periods of humid atmospheric conditions, the number of leaf and vine lesions was very large, the leaf spots enlarged very rapidly and in many instances the enlarging spots coalesced and formed great patches of necrotic tissues. Such rapidly enlarging spots failed in many occasions to form the characteristic reddish halo observed in lesions with arrested growth.



FIG. 1. Natural infections on cuttings of *Basella rubra* L. showing leaf and vine lesions caused by *Acrothecium basellae*. Size greatly reduced.

Leaf lesions were frequently observed developing near the leaf margins, and particularly near the tips. If near each other, the lesions coalesced and enlarged toward the midrib. With the progress of the disease the leaf margins became ragged. Severely affected leaves became chlorotic, the yellowing invading nearby necrotic leaf areas and extending throughout the blade. Such severely infected leaves abscised. Affected leaves showed pronounced curling and sometimes rolling. Young as well as mature leaves were found very susceptible to the disease, although more so in muggy weather (Fig. 1).

Vines of infected plants at first showed very small reddish lesions similar

to those already described occurring on leaves. In dry weather, the lesions enlarged longitudinally, forming long, reddish spots which in very few cases girdled the vines. The lesions were lustrous, reddish and dry, very superficial. The parasite confined its parasitism almost entirely to the endodermal tissues of the vines, except in very muggy weather when the internal vine tissues were also involved. Under such cases of extreme humidity and hot temperature, the vine lesions were also found invaded by secondary soil organisms. A *Rhizoctonia* sp. was frequently isolated from *Acrothecium* lesions at the base of the vines or on vines running over the ground (Fig. 2).



FIG. 2. Advanced lesions at base of vine of *Basella rubra*, caused by *Acrothecium basellae*. Natural size.

The secondary invasion of *Acrothecium* vine lesions by the saprophytic *Rhizoctonia* sp. culminated in a soft, mushy rot of the vines at the points of infection. Eventually the whole vines dried up.

#### THE FUNGUS

*Taxonomy.* The parasite is a species of *Acrothecium*, a Deuteromycete belonging to the group Dematiaceae of the Fragmosporeae (Fig. 3). This is the fifth record of an *Acrothecium* from Puerto Rico. In 1919, Tehon (9) described a fungus on *Setaria* sp., as *A. flacatum*. In 1930, Ashford and Ciferri (1) described under the name of *A. obovatum subcapitulatum* an organism growing saprophytically on human skin. Toro, in 1926, described the fungus *A. polytriades* occurring on leaves of *Polytriades amauris* L. *A. capsici* Turconi, was reported on *Capsicum frutescens* L.

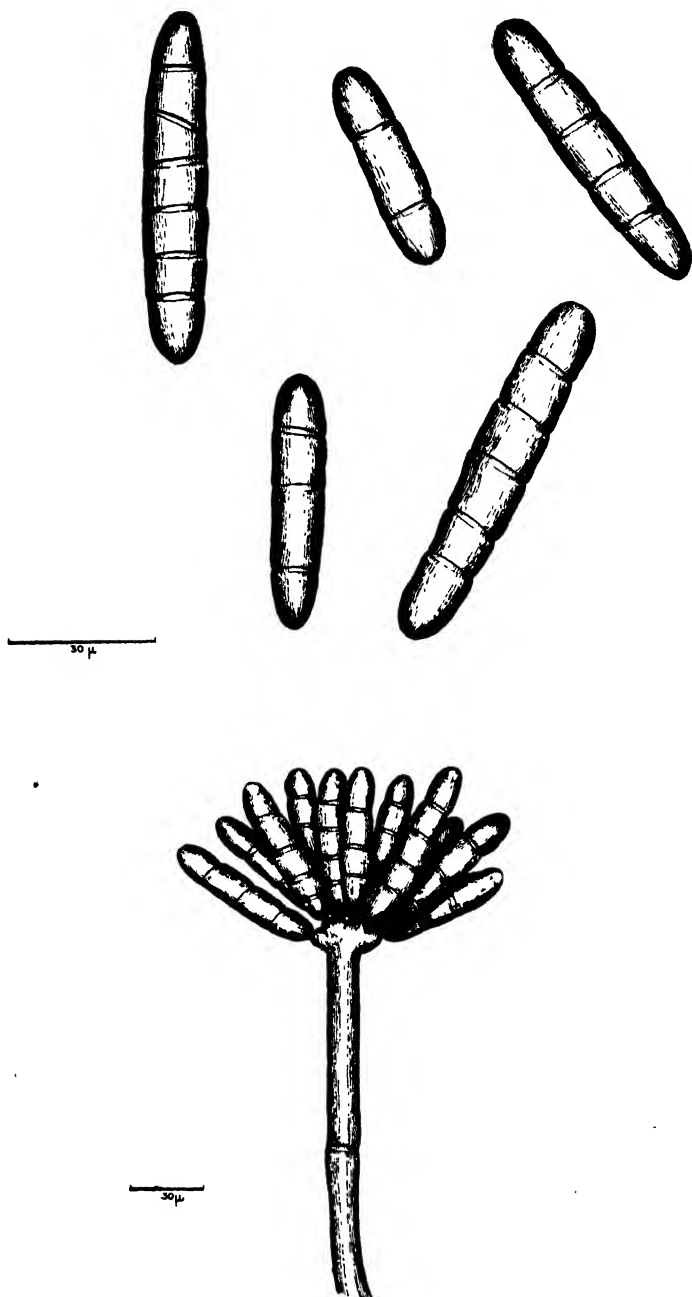


FIG. 3. Conidia of *Acrothecium basellae* and conidiophore the latter showing manner of sporulation. Material obtained from a pure culture of the organism on potato-dextrose-agar kept at 30°C., Drawing made with camera lucida.

Other organisms reported outside Puerto Rico are: *A. nigrum*, *A. obovatum* as a saprophyte on wood, *A. lunatum* Wak., growing saprophytically on sugarcane leaves, also parasitic on *Panicum frumetaceum*, *Eleusine corocana* and *Setaria italica* in India, and *A. penniseti* responsible for a leaf spot of *Pennisetum T'ypoideum*. The morphological characters of all these organisms are different from those of the parasite described in this paper, particularly in shape and size of spores and the formation of sclerotia of the latter, a characteristic which appears to be reported here for the first time for this genus.

Many authors have recognized the necessity of revising the genus *Acrothecium*. They substantiate their criteria on the basis of morphological variations of organisms grouped in this genus; variations that participate in characteristics of other genera. Ashford and Ciferri (1) reported variation in cultures of *A. obovatum subcapitulatum* corresponding to such other genera as *Brachysporium*, *Spondylocadium* and *Napicladium*. They also reported that uniseptate conidia resembled *Cordana* and continuous conidia *Acrotheca*.

Tehon (9) found similar behavior in sporulation and other characters in culture of *A. flacatum*. Manoranjan Mitra (5) also observed in cultures of *A. penniseti* the formation of acrogenous conidia and group of conidia below the apex of the conidiophores. *A. basellae*, the parasite on *B. rubra* and the subject of this paper, produced only acrogenous, cylindric spores, conforming in these and other particulars very closely to the general characters ascribed to the genus *Acrothecium*.

*Morphology of the fungus on the host:* The mycelium consisted of subhyaline hyphae which ramified inside the host tissues, intra and intercellularly, involving the entire leaf tissues. In the vines, the mycelium was confined mostly to the endodermal cells.

Conidiophores were produced amphigenously in the central necrotic tissues of the leaf lesions, arising from sub-stomatal, sclerotia-like bodies or pushing out to the surface between dead cells. In dry conditions, few conidiophores were observed on the leaf lesions, but they were very abundant when the relative humidity of the atmosphere was high. The conidiophores were solitary, rigid or slightly bent, long-septate, very long, sometimes reaching a length of 500  $\mu$  or even longer. In width, the conidiophores varied from 9 to 15  $\mu$ . The conidiophores were subhyaline when young and not particularly differentiated from the fruiting mycelium. In old cultures, the conidiophores turned brownish. At the apex of the long conidiophores tubercle-like sterigmata were produced in variable numbers, depending on the degree of sporulation which had occurred at the time of microscopic observation. Sterigmata when in large number, formed very conspicuous grape-like clusters. Sterigmata fluctuated from 9 to 15  $\mu$  in diameter.



Conidia were produced acrogenously, single or fasciculate as outgrowths from the sterigmata. At first, the conidia were subhyaline projections which enlarged to become long, cylindric, dark, olive-brown spores measuring from 18 to 92  $\mu$  in length and from 7.5 to 15  $\mu$  in width. The acrogenous conidia were found in variable number, fluctuating from 1 to 15 or perhaps more, arranged in fascicles at the apex of the conidiophores. Due to the lack of mucilage formation at the head of the conidiophores, the conidia detached easily when blowing a soft current of air over the cultures or when floating the conidiophores and their conidia in water. Conidia formation was very profuse in humid conditions. In dry conditions sporulation was not appreciable. Sporulation was easily induced when infected leaves were placed in moist chambers, spores being formed overnight.

Microscopic examination of infected leaves from plants growing in moist environment revealed a profuse mycelial development of the parasite inside the infected leaf tissues. Abundant conidia formation was noticed at the head of numerous subhyaline or dark conidiophores. Sclerotia formation was abundant in the necrotic tissues of the lesions. The sclerotia were dark, semi-hard, composed of anastomosed, thick wall hyphae, were relatively small and not enlarging beyond 1 mm. in diameter.

#### CULTURES

Pure cultures of the *Acrothecium* sp. were obtained by pouring dilution plates with conidia washed from infected leaves kept in moist chambers; thus inducing abundant sporulation. Owing to the large size of the spores, fishing them out of the cultures to make subcultures was an easy matter.

*Germination of conidia in distilled water.* Conidia placed in distilled, sterile water germinated readily within an hour or two. Germination tubes were extruded from apical and basal cells as well as from intermediate cells of the spores. One or more germ tubes were produced either from one or from various cells of the spores. The germ tubes grew very rapidly until the spore was disorganized (Fig. 4, A).

*Morphology of the fungus in cultures.* The parasite grew well on different culture media. Nutrient-2% dextrose-agar was found very satisfactory for growth and sporulation. On this medium the mycelial growth was profuse, provided the pH values were around neutrality and the temperature relatively high. Growth of the mycelium was mostly submerged, radial subhyaline at first and later dark (Fig. 4, B, C). The substratum changed gradually to brownish.

Sclerotia formation was very profuse, and those formed were imbedded in the medium. At the start of their formation the sclerotia were subhyaline, becoming dark brown at maturity; they were almost homogeneous in structure and shade, not smooth, usually single, sometimes conglom-

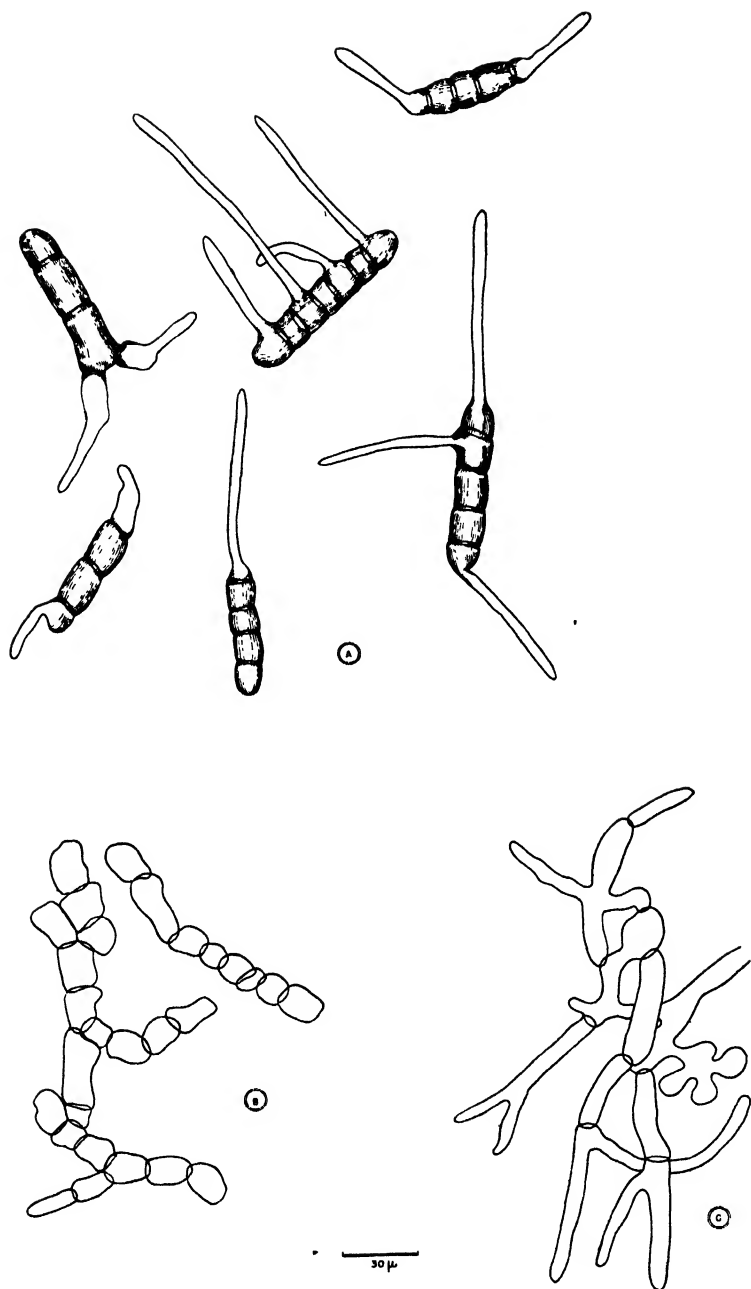
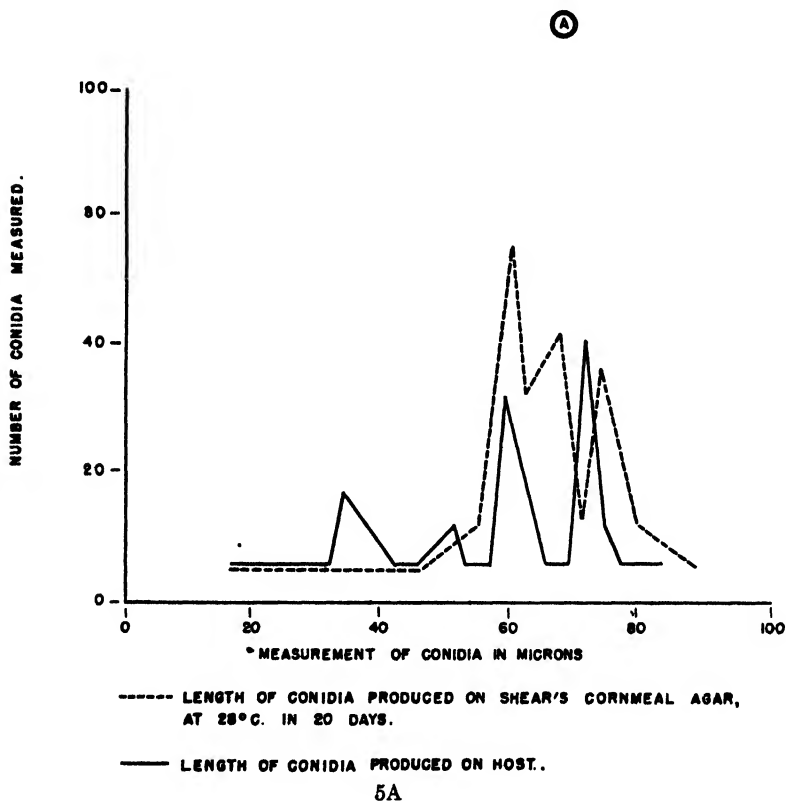


FIG. 4. Above, conidia of *Acrothecium basellae* germinating within three hours of being immersed in distilled water. Below, close-septate and long-septate mycelium of organism in culture.

erated and arranged radially following the growth of the mycelium. Sclerotia fluctuated in size from 160 to 320  $\mu$  in diameter, were subspherical or irregularly shaped, semi-hard.

Sporulation was very abundant, conidia being produced apically in fascicle and corresponding in size and number with those observed on natural leaf lesions. Conidia varied from 30 to 72  $\mu$  by 9 to 15  $\mu$ , were



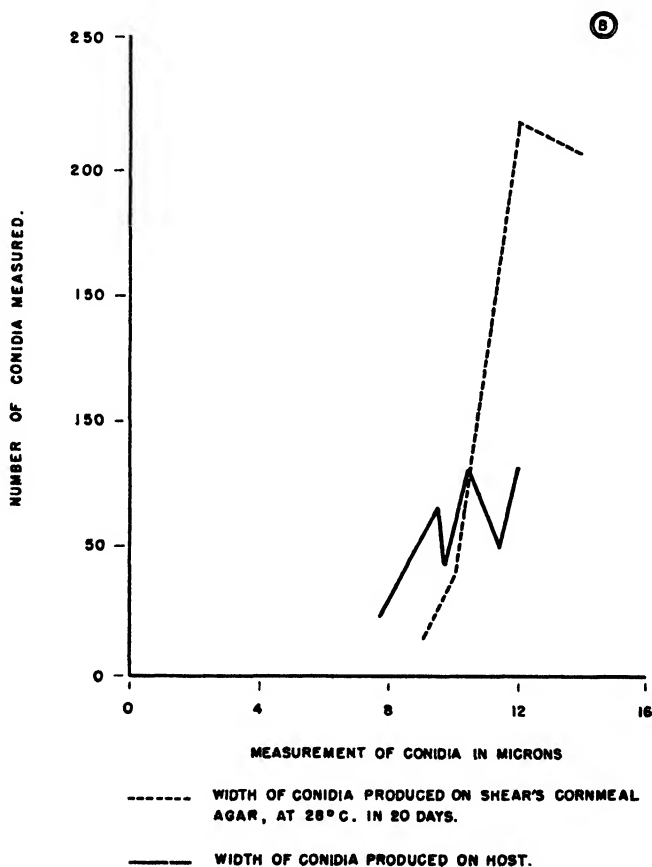
generally three-septate to five-septate and slightly constricted at the septa. In old cultures the hyphae became thick-walled, forming chlamydospores.

*Growth of parasite on other media.* On 2% dextrose-agar with an acid reaction of 6.71 and kept at 30°C., the growth was rather poor, superficial, mostly submerged, radial, subhyaline at first, later olive-brown. Few sclerotia were formed, ranging in size from 120 to 320  $\mu$ . Sporulation was also poor or did not occur at all in some tubes. Spores were within range of spore measurements observed in nature,

On 2% dextrine-agar, pH 6.37, kept at 30°C., the growth was scanty,

submerged, subhyaline or slightly superficial, turning later olive-brown. Sclerotia varied in size from 108 to 196  $\mu$ . Few spores were produced.

On cornmeal (Shear's) agar, pH 6.14, kept at 30°C., the mycelium was submerged or slightly superficial, scattering and radially arranged, closely septate and slightly constricted at the septa. Width of hyphae of submerged mycelium varied from 6 to 9  $\mu$ , septation occurred every 15 to 30  $\mu$ .



5B

FIG. 5. Comparison of size of spores of *Acrothecium basellae* produced on the host and on artificial substratum.

Conidia formation was relatively abundant; conidia were one to five-septate, granular, and from 24 to 81  $\mu$  in length by 9 to 15  $\mu$  in width. Conidiophores were very long, slightly tinged brown, not abundant, and rather scattered on the medium. Sclerotia usually were produced rather singly, few in number and relatively small, varying from 45 to 105  $\mu$  in size (Fig. 5).

On 2% maltose-agar, pH 5.64, kept at 30°C., mycelial growth was slow, submerged and olive-brown. Spore formation was poor. Sclerotia were abundant, agglomerated, rather small.

*Relation of pH values of the substratum and growth.* Several lots of 2% dextrose-nutrient-agar plates from the same batch, were treated with lactic acid and adjusted to varying pH values. A range from pH 4.27 to pH 7.15 was prepared; the reactions were determined by means of a potentiometer. Four plates of each set of pH values were respectively planted with mycelial disks, 0.5 cm. in diameter, of the same age. Twenty four hours

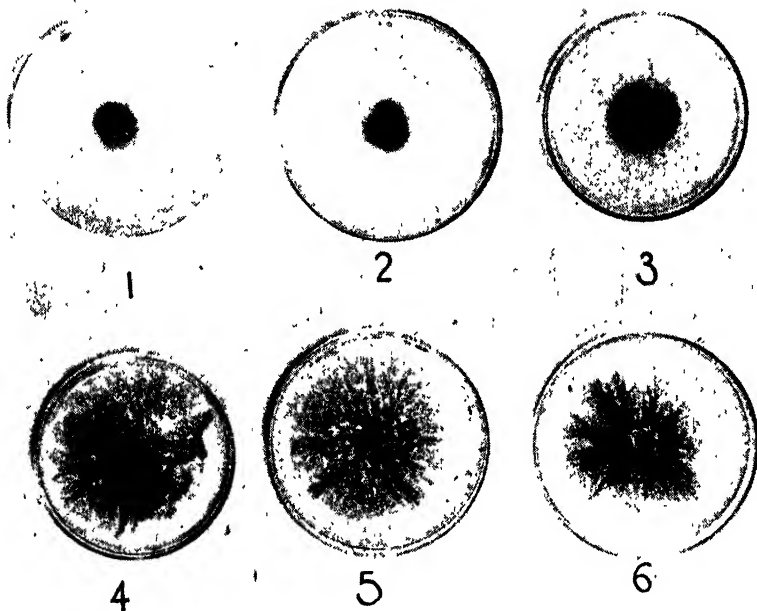


FIG. 6 Growth of *Acrothecium basellae* in six days on dextrose-peptone-agar adjusted to pH values of 4.27, 4.61, 5.29, 5.97, 6.47, 7.15, respectively. Cultures kept at 30°C. Note abundance of sclerotia in plates 4, 5, and 6.

increments of growth of each colony were recorded for a period of seven days. The records represented the average of two diameters of each colony taken at right angles to each other. The average of each set of four plates was jotted down as the growth rate at a given pH value. The results obtained showed that the parasite was favored by slight acid or alkaline reaction of the substratum (Fig. 6).

*Relation of temperature and growth.* Four plates containing 2% dextrose-nutrient-agar, pH 7.15, were respectively planted with 0.5 cm. in D. mycelial disks taken from young, actively growing cultures of the parasite.

The plates were kept for twelve hours at 28°C., until the colonies have enlarged 2 mm. in radial growth. The cultures were subsequently placed in lots of four in incubators set at 0, 9, 20, 24, 28, 32, 35 and 37°C. The growth rate per day of each colony was recorded as previously explained. The results obtained indicated that high temperatures favored the growth

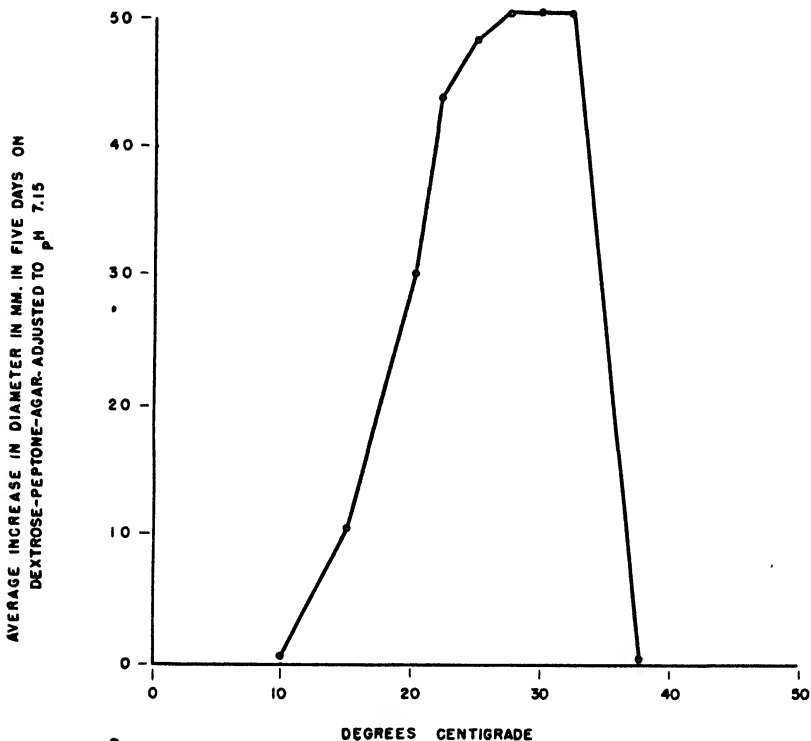


FIG. 7. Relation of temperature to growth of *Acrothecium basellae*

of the parasite. The fastest growth occurred at temperatures within 28 to 32°C. (Fig. 7).

In view of the fact that a parasite of the genus *Acrothecium* attacking *Basella rubra* L. has not been previously reported in American and probably not in Eurasian and African literature, the present fungus is described here as a new species:

*Acrothecium basellae* sp. nov.:

Conidiophores amphigenous, rigid, erect or subdecumbent, simple, 3 to 5 septate, solitary or sometimes fasciculate; apex swollen with numerous tubercle-like sterig-

mata, subhyaline at first, later olive-brown, not differentiated from the fertile hyphae, except for its long septation and swollen head, length of conidiophores more or less 500  $\mu$ . Conidia acrogenous, cylindric, olive-brown, single, produced as an outgrowth of sterigmata, numerous, in groups of up to 15 or more, at apex of conidiophores, easily detached, 1 to 7 septate, slightly constricted at septa, varying in length from 18 to 92  $\mu$  by 7.5 to 15  $\mu$  in width, thick-walled. Sclerotia numerous in necrotic tissues of leaves and vines, subepidermal, imbedded in substrata, varying in diameter from 40 to 1000  $\mu$ , irregularly shaped, dark, semi-hard, not smooth.

#### PATHOGENICITY

Observations of artificial inoculation with conidia obtained from pure cultures of the parasite and from natural infections on leaves, followed by reisolations of the parasite, showed the constant association of the *Acrothecium* sp. in question and the disease.

Inoculations were conducted in a greenhouse with an air temperature fluctuating from 24°C. to 37°C., and outside under a half lath-shade plant house with an air temperature ranging from 26 to 30°C. Cuttings of *B. rubra* apparently free from natural infection, were obtained and rinsed several times under a faucet to remove dirt and possible conidia of the parasite that might have landed on them. The cuttings were placed with cut ends immersed in distilled water and in a three-salt nutrient solution. In this manner they were left for four weeks in a humid environment to allow for germination of conidia of the parasite if present on the leaves, and infection.

In August, the disease-free cuttings having several leaves, were inoculated with a suspension of conidia of the parasite taken from pure cultures of the organism. The conidia were sprayed over the leaves and vines of the cuttings with a De Vries atomizer. The cuttings after spraying were kept inside a large glass box with adjustable side doors and with inside shelves for keeping a thermograph and a hygrograph. The air temperature inside the glass box fluctuated from 26 to 35°C. daily and the relative humidity was around 80%. Control plants were similarly treated but not inoculated, spraying being done with distilled water only. Twelve cuttings were used in this test. A parallel test was conducted outside under the half-shade plant house.

Within a week after inoculation, the cuttings kept in the glass box showed symptoms of disease. Small yellowish lesions were evident on the leaves (Fig. 8). Seven days later, small reddish lesions were also evident on the vines. Cuttings kept outside under the lath-shade plant house were also showing the same symptoms of disease. The weather outside has been rather hot, 30°C. in the shade, and the relative humidity very high, sometimes around 80%, due to the frequency of rains and numerous cloudy days.

After infection was noticed on the inoculated cuttings, half of them were taken out of the glass box and kept in a greenhouse with a relative humidity around 40%, and a temperature range of 26°C. to 35°C. daily.

Observations of the infected cuttings kept inside the glass box and those in the greenhouse, showed that relative humidity of the environment is the most important factor for disease. The cuttings in the glass box showed constant enlargement of the leaf and vine lesions, culminating in blight. Cuttings outside the box in the greenhouse showed arrested development of lesions and a complete check of further infection and blight. It appeared therefore that under natural conditions the organism isolated and found parasitic on *B. rubra*, is favored by high relative humidity and high temperature. During the summer of 1943, the weather was hot and humid



FIG. 8. Artificially infected leaves of *Basella rubra* L., showing characteristic lesions produced by *Acrothecium basellae*. Uninoculated leaf at left.  $\times \frac{1}{2}$ .

conditions which undoubtedly favored the rapid spread of the disease. Abundant precipitation occurred and the temperature was high, the days were cloudy and muggy.

Plants in the WPA garden were grown on trellis and in an exposed place, which though windy, was particularly humid due to the humid air coming from nearby hills. In this environment very copious sporulation occurred as shown by periodic microscopic examination of leaves. Periodic examination of recently infected leaves also revealed the manner of fungal penetration of healthy tissues. Penetration was observed to occur either through the cells or between the cells or by way of stomata. In some of the germinating spores, appressoria-like structures were noticed before penetrating pecks were produced.

Conidia were evidently blown from place to place. Infections occurred



more abundantly on the leeward side of the field. Leaf lesions were very abundant in mature and in newly formed leaves.

Microscopic examination of infected leaves that had fallen on the ground revealed the profuseness of sclerotia formation in the dead leaf tissues. It appears, therefore, that the parasite is able to pass its saprogenic cycle in the soil in the form of sclerotia. With the advent of favorable weather conditions these sclerotia are liable to germinate and sporulate. When dead leaves with abundant sclerotia were placed in moist chambers the latter were found to germinate. The mycelial growth sporulated in few days. This tends to confirm the supposition that saprogenesis occurs in nature in the form of sclerotia.

#### CONTROL

The value of spraying with Bordeaux was determined before attempting to try other fungicides. Weekly spraying with Bordeaux formulae 3-3-50 and 4-4-50, proved very satisfactory in controlling the disease. Best results were obtained with the stronger formula. Spraying was initiated on September and continued until November. The summer was very humid and hot, characterized by frequent rainy and cloudy days.

One row of plants, approximately 24 feet long was selected on the leeward side of the infected field. In this manner there was a good chance for a constant blowing of conidia from the infected windward side of the field. At the end of eight weeks of weekly sprayings new shoots and leaves were produced almost free from infection. Unsprayed plants left as control on the same row, showed abundant leaf and vine lesions.

The disease was found to be checked by dry spells. Relative humidity is considered the most important factor determining the incidence of the disease in Puerto Rico, where high temperatures are registered the year round. Therefore, spraying was necessary only during humid weather. Plowing under infected plant debris, and crop rotation are also recommendations which might help in the prevention of the disease.

#### SUMMARY

A leaf spot of *Basella rubra* L., caused by an *Acrothecium* species, not previously recognized in Puerto Rico nor probably elsewhere as a parasite of the above mentioned plant, is described in this paper. The parasite was found to produce leaf lesions within 5 to 7 days after inoculation with conidia obtained from pure cultures of the organism. Vine lesions were somewhat delayed in their appearance.

Humid atmospheric condition accompanied by relatively high temperature, favored the incidence of the disease, as shown by pathogenicity tests conducted under glass house and field conditions. The foliage of heavily

infected plants blighted in humid environment and became useless for consumption.

Weekly sprayings with Bordeaux, 3-3-50 and 4-4-50, were found successful in controlling the disease, the latter formula giving better results under persistent humid conditions of the environment.

#### RESUMEN EN ESPAÑOL

Durante el verano del 1943 se observó en una siembra de Espinacas del País (*Basella rubra* L.) una chamusquina de las hojas, apareciendo ésta primeramente en forma de pequeñas manchas rojizas que al agrandarse y venir en contacto formaban mancharon de tejidos necróticos descoloridos.

El ambiente en que crecían dichas plantas demostró ser propicio para el desarrollo de la enfermedad. El tiempo fué húmedo, cálido y los días se presentaron lluviosos y generalmente nublados. Un examen microscópico de las lesiones en las hojas reveló la abundancia de conidióforos extremadamente largos y rematados en esterígmata en forma de ramilletes de uvas. De estas esterígmata produjéronse esporas alargadas, cilíndricas, tabicadas hasta 7 veces, trasversalmente, y formando en conjunto un ramillete. La morfología de este organismo demostró pertenecer al género *Acrothecium*. Por considerarse una especie hasta el presente no informada en *B. rubra*, se describe en este trabajo como una especie nueva, dándosele el nombre de *Acrothecium basellae*.

Al hacerse cultivos con esporas tomadas de lesiones en las hojas, como así también de esclerocios tomados de tejidos necróticos en dichas hojas, se aisló invariablemente el susodicho organismo. Pruebas de patogenicidad realizadas con conidios del organismo producidos en cultivo puro demostraron incontrovertiblemente su virulencia y única relación con la enfermedad en cuestión. Los síntomas producidos fueron aquéllos anteriormente observados ocurriendo naturalmente en las hojas. El examen microscópico de las lesiones producidas por inoculación artificial con conidios tomados de cultivos puros, reveló la presencia del organismo en los tejidos parasitados y la morfología del mismo vino a corroborar su identidad como el que fuera aislado repetidamente de lesiones naturales en las hojas. Al hacerse cultivos de tejidos de lesiones de hojas artificialmente inoculadas se recuperó invariablemente el parásito.

El examen necroscópico de las lesiones en las hojas mostró la presencia de abundantes esclerocios en los tejidos subepidermales. Las observaciones hechas hacen creer que el parásito perdura saprogénicamente en residuos de hojas y tallos enfermos. Cuando el ambiente es propicio germinan dichos esclerocios; producen nuevamente conidios y la enfermedad vuelve a aparecer.

Ensayos realizados con caldo bordelés (fórmulas 3-3-50 y 4-4-50)

demostraron que la enfermedad se puede combatir efectivamente con este fungicida. La fórmula más concentrada dió mejores resultados, particularmente en tiempo extremadamente húmedo. Las pulverizaciones se espaciaron cada 7 días, encontrándose que así repartidas eran suficientes para combatir la enfermedad.

Pruebas hechas en invernáculos demostraron como, en un ambiente marcadamente seco (40% de humedad relativa), se retardaba marcadamente el aumento en tamaño de las lesiones y disminuía el número de éstas. En tiempo marcadamente seco no es de esperarse que sea tan severa la enfermedad; bastaría, si fuere necesario, pulverizar con bordelés de vez en cuando.

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# ALTERNARIA BLIGHT OF BACHELOR'S BUTTON (*GOMPHRENA GLOBOSA* L.)

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During the hot, humid summer of 1937, the writer observed a severe case of leaf and stem blight of the ornamental plant commonly known as Bachelor's Button (*Gomphrena globosa* L.), in flower gardens in the neighborhood of Río Piedras. Outbreaks of the blight were later observed throughout the Island. In every instance an *Alternaria* species was isolated from affected leaf and stem tissues of diseased plants collected in flower gardens. A similar fungus was also isolated from the wild species *G. dispersa* Standley. Pathogenicity test with these fungi showed that both organisms were one and the same species. The purple, white and red varieties of *G. globosa* were found to be equally susceptible to the disease.

Up to the present and to the author's knowledge, there has never been reported in Puerto Rico a fungus of the genus *Alternaria* attacking *Gomphrena* species. Apparently there is no record of such occurrence in American literature.

In Japan, Togashii (1) reported a severe blight of *G. globosa* and showed by artificial inoculation with conidia of an *Alternaria* species, that this fungus was the causal agent of the disease. No infection, however, was obtained when inoculating with conidia obtained from pure cultures of the fungus. Yoshii (2) also reported a similar organism, resembling morphologically *A. gomphrenae* Togashii responsible for a leaf spot of *G. globosa*. The organism however, is claimed to be different to that previously reported by Togashii as far as cultural characters are concerned. Both species of *Alternaria* produced similar clinical syndroms, characterized by dark reddish purple spots with smoky-gray necrotic centers.

The leaf symptoms of the *Alternaria* blight observed in Puerto Rico correspond with those described in Japan. The morphology and physiology of the *Alternaria* species prevalent in Puerto Rico seems to justify our assumption that this fungus is the same species already reported and described in Japan by Togashii.

## THE DISEASE

The first noticeable symptoms of the disease under our environmental conditions were the appearance of small (0.25–0.50 mm. in diameter) yellowish-green spots, visible on both sides of the leaves, although more conspicuously on the upper side. These spots were produced more rapidly

and abundantly on the lower leaves. As the spots enlarged and grew older, they turned yellowish-brown, forming concentric rings of varying shades of that color. The innermost necrotic tissues became discolored and translucent. The invaded tissues around the yellowish-green spots turned first dull green and with the advance of the disease became discolored. When the lesions seemed to have finished their enlargement, a reddish halo was formed encircling the yellowish-brown spot. This halo was characteristic of the disease and did not fade out except when the lesions were very old and the leaf had turned light brown. In some spots, for some reason, the halo was not formed or was very pale. In old lesions although the halo had vanished, the affected necrotic areas were sharply defined. The spots appeared scattered all over the leaf surfaces and were of a sub-circular outline, except when they occurred near the margins or near the veins, in which case they became irregular. Nearby spots enlarged, coalesced, and envolved a great part of the leaf. With the progress of the disease, the leaves corrugated and rolled, becoming finally dirty brown throughout and were found hanging down along the stems. They stayed in this position for several days and then dropped. The lesions on the stems were very similar to those found on the leaves. However, on the stems they were elongated. Some of the stem lesions on becoming old lost their dahlia-carmine halo and a whitish discolored area remained. These lesions enlarged, coalesced and girdled the plant.

#### THE PATHOGENE

*Methods of isolation.* During the summer of 1937, the fungus was isolated from fresh, young lesions on leaves and stems of the purple variety of *G. globosa* L., by planting pieces of diseased tissues, previously disinfected for 2-3 minutes in a 1-1,000 solution of bichloride of mercury and afterwards washed several times in sterilized water, on agar plates and from dilution plates of conidia washed from young lesions.

*The organism in culture.* The fungus grew well in potato-dextrose agar, prune agar, bean agar, Cook's No. 2 agar, and oatmeal agar. In oatmeal agar, the organism formed aerial, cottony, white to light gray mycelium and olive-brown or ochreous trophic and fructiferous growth. The non-fertile hyphae were elongate-septate, minutely guttulate and with septa spaced from 25 to 83  $\mu$  and slightly constricted. Fertile hyphae were recognized by the toruloideous sub-fasciculate shape, by the presence of large, refractive oil globules which in some instances filled the lumen of the cell and by the closeness of the septa, spaced from 3.6 to 7.2  $\mu$ . These fertile hyphae were definitely light brown or ochreous in color, and were found to produce numerous chlamydospore like bodies. The substratum of the media and particularly those with a high content of carbohydrates

was tinged dark brown or ochreous. Sporulation occurred on oatmeal agar and in small amount. The conidiophores as well as the conidia were formed from protuberances of the cells and were hyaline at first. Later, at maturity, they turned light-brown or ochreous. No sporulation took place in other culture media.

*Morphology on the host.* The fungus once it had entered the host, ramified and spread in the host tissues. The hyaline hypha, of approximately  $8\ \mu$  in thickness, was septate and slightly constricted at the septa. Eventually, the invaded tissues were killed, became necrotic, and of a yellowish-brown color. The conidiophores were produced amphigenously, although more abundantly on the upper side of the leaf, and particularly along the yellowish-brown area next to the dahlia-carmine halo. These conidiophores were produced singly or in thin tufts, erect or subdecumbent; were 1 to 5 septate and from 35 to 70 by 4 to  $5\ \mu$  in width and emerged by way of the stomata or pushed their way through the necrotic areas of the lesions. The conidia, formed generally singly at the apex of the conidiophores, were easily detached, being elongate, obclavate, with basal and blunt or pointed, 1 to 9 septate, and slightly constricted at the septa; longitudinal septa few or lacking, granular or with refractive oil globules, 60 to 160 by 12 to  $16\ \mu$ , long beaked, never branched, septated every 10 to  $15\ \mu$  and ranged from one to three times the length of the broadest part of the spore.

#### PATHOGENICITY

Plants grown from seeds in sterilized soil were inoculated on August 8, 1937 in the afternoon of a cloudy day with a suspension of spores washed from the typical lesions and from pure culture, respectively. Two sets of plants were inoculated by means of a DeVries atomizer. No lesions were made to the leaves, stems or branches. Half of the plants from each group were covered with bell jars and the others were left uncovered. Control plants were similarly treated but not inoculated. Three days later, typical tiny yellowish green, spots were evident. Microscopic examination of the matured lesions showed the constant association of the pathogene. Re-isolations from the newly formed lesions ratified the pathogenicity of the organism. Conidia were found to be produced within 4 to 6 days after inoculation.

#### EPIPHYTOLOGY

High temperature combined with ample atmospheric moisture seemed to favor the development of the parasite. Under such environmental conditions spores were produced abundantly on leaf lesions. Spores are spattered by rain drops or carried by wind. New cases of *Alternaria* blight appeared on plants where the spores have landed. Saprogenesis appeared to occur in debris of diseased plants.

## CONTROL

Eradication of dead or severely diseased plants, as well as the destruction of all diseased material by deep plowing or by burning, clean cultivation, good drainage and planting during the dry season will contribute in checking the disease.

## SUMMARY

1. An *Alternaria* sp. is reported occurring on *Gomphrena globosa* L. and *G. dispersa* Standley.

2. All the ornamental varieties as well as the wild type are susceptible to the disease.

3. The disease is recognized by the sub-circular, yellowish-brown lesions on stems and leaves with a characteristic reddish halo.

4. The pathogene, an *Alternaria* sp. is described.

5. Conidia from young lesions as well as from an oatmeal culture were used for morphological, physiological and inoculation purposes.

6. The inoculation tests with spore suspensions taken respectively from young lesions as well as from culture proved the pathogenicity of the organism.

7. Conidia placed in water and at room temperature (28 C.) started germination within an interval of 2 to 3 hours.

8. Sporulation was noticed on lesions on the leaves and never on the stems. Dead parts of the plants seem to harbor the pathogene.

9. The disease is more severe during hot, rainy weather.

## RESUMEN EN ESPAÑOL

Durante el verano de 1937, especialmente en la vecindad de Río Piedras, se pudo observar la difusión de una enfermedad atacando las hojas y las ramas de las siemprevivas (*Gomphrena globosa* L. y *G. dispersa* Standley). Se presenta esta enfermedad en las hojas inicialmente con manchas esparcidas, rara vez confluentes, pequeñas de 0.25 a 0.50 mm. de diámetro, circulares, amarillentas y bordeadas por una zona verde mate. Las lesiones al aumentar de tamaño pueden ser irregulares, confluentes y presentan los tejidos centrales necróticos y de color pardo claro y rodeados muy claramente por un área de color rojizo, persistente. Las hojas atacadas se deforman, mueren, cayéndose finalmente. En los tallos las lesiones muestran características similares, pero son generalmente irregulares, alargadas y unilaterales, pero en ciertas ocasiones son confluentes, rodeando el tallo y produciendo la muerte de la parte superior.

Un examen microscópico de las lesiones muestra los tejidos serpenteados por el micelio tabicado de un hongo de donde emergen, sea a través de los

tejidos o por estomas, los hacecillos de conidioforos simples, oscuros, continuos o tabicados, al extremo de los cuales se forman esporas mazudas, oliváceas y acres, tabicadas longitudinal y transversalmente, lo que cataloga a este organismo patógeno entre los hongos del género *Alternaria*. Siendo esta especie un nuevo record para Puerto Rico, se describe a continuación:

*Alternaria gomphrenae* Togashii. En nuestro ambiente este hongo produce conidióforos oscuros, tabicados una o cinco veces transversalmente, solitarios o fasciculados, erguidos o inclinados, simples o ramosos. Los conidios oliváceos u ocre, mazudos, con el ápice extremadamente alargado y fino, con 1 ó 9 tabiques transversales y con o ningún tabique longitudinal; el contenido granular u oleaginoso, de 60 a 160 por 12 a 16  $\mu$ . en tamaño.

La enfermedad se manifiesta mayormente en épocas de lluvias frecuentes y en sitios donde hay poca aereación y mal avenamiento.

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## A METHOD FOR THE SOLUTION OF NORMAL EQUATIONS

By BERNARDO G. CAPÓ

*Biometrician*

### INTRODUCTION

The methods employed for the solution of a set of normal equations have been modified in recent years to employ modern calculating machines to the fullest extent. Remarkable among these modifications is the abbreviated Doolittle solution due to Dwyer (2), which requires a considerably reduced number of entries in the table of solution of such a system of equations. Dwyer (2, p. 443) claims that this method "is unquestionably the method which should be used by those seeking numerical solutions to the classical problems of multiple and partial correlation." Hotelling's experience with this and other methods has led him to state (4, p. 16) that "for the mass of least-square and other problems in which the inverse of a matrix is needed, the best procedure appears to begin with one of the methods described by Dwyer," etc. It is the purpose of this article to call attention to a method which is simpler, at least for relatively small numbers of constants to be fitted, than Dwyer's abbreviation of the Doolittle method.

### THEORY OF THE METHOD

The method is based on Laplace's development of a determinant, stated by Bôcher (1, p. 26) as follows:

"Pick out any  $m$  rows (or columns) from a determinant  $D$ , and form all the  $m$ -rowed determinants from this matrix. The sum of the products of each of these minors by its algebraic complement is the value of  $D$ ."

The application of this principle to the solution of a set of normal equations will be explained now.

Let

$$A_{123\dots n} = \begin{vmatrix} a_1 & b_1 & c_1 & \cdots & m_1 \\ a_2 & b_2 & c_2 & \cdots & m_2 \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ a_n & b_n & c_n & \cdots & m_n \end{vmatrix}, \text{ and let, for } i < j < k, \text{ and with reference to } A_{123\dots n},$$

$$A_{ij} = \begin{vmatrix} a_i & b_i \\ a_j & b_j \end{vmatrix} = a_i b_j - a_j b_i,$$



for example,  $A_{25} = \begin{vmatrix} a_2 & b_2 \\ a_5 & b_5 \end{vmatrix} = a_2b_5 - a_5b_2;$

$$B_{ij} = \begin{vmatrix} b_i & c_i \\ b_j & c_j \end{vmatrix} = b_ic_j - b_jc_i,$$

for example,  $B_{24} = \begin{vmatrix} b_2 & c_2 \\ b_4 & c_4 \end{vmatrix} = b_2c_4 - b_4c_2;$

$$A_{ijk} = \begin{vmatrix} a_i & b_i & c_i \\ a_j & b_j & c_j \\ a_k & b_k & c_k \end{vmatrix}; \quad \text{for example, } A_{146} = \begin{vmatrix} a_1 & b_1 & c_1 \\ a_4 & b_4 & c_4 \\ a_6 & b_6 & c_6 \end{vmatrix};$$

$$A_{ioj} = \begin{vmatrix} a_i & c_i \\ a_j & c_j \end{vmatrix} = a_ic_j - a_jc_i,$$

for example,  $A_{206} = \begin{vmatrix} a_2 & c_2 \\ a_6 & c_6 \end{vmatrix} = a_2c_6 - a_6c_2;$  etc.

Now, the application of the above-mentioned principle will show that

$$A_{123} = A_{12}c_3 - A_{13}c_2 + A_{23}c_1$$

$$A_{1234} = A_{123}d_4 - A_{124}d_3 + A_{134}d_2 - A_{234}d_1, \text{ etc.}$$

The sign of any given term will be positive if "0" or an even number of inversions of adjacent subscripts is required to bring them into an ascending order, and negative if said number of inversions is odd.

Fisher's (3, p. 144) modification of Gauss' (5, p. 60 and 84) method of undetermined multipliers applied to the set of normal equations:

$$Aa_1 + Bb_1 + \cdots + Mm_1 = \text{Say,}$$

$$Aa_2 + Bb_2 + \cdots + Mm_2 = \text{Sby,}$$

$$\begin{array}{ccc} \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{array}$$

and  $Aa_n + Bb_n + \cdots + Mm_n = \text{Smy,}$

where  $a_1 = Sa^2$ ,  $b_1 = a_2 = Sab$ ,  $\cdots$ ,  $b_2 = Sb^2$ ,  $c_2 = b_3 = Sbc$ ; etc., requires the solution of the following sets of equations for  $C_{aa}$ ,  $C_{ab}$ ,  $\cdots$ ,  $C_{bb}$ ,  $C_{bc}$ ,  $\cdots$ , and  $C_{mm}$ :

$$\text{1st set: } C_{aa}a_1 + C_{ab}b_1 + \cdots + C_{am}m_1 = 1$$

$$C_{aa}a_2 + C_{ab}b_2 + \cdots + C_{am}m_2 = 0$$

$$\begin{array}{cccc} \vdots & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & \vdots \end{array}$$

$$C_{aa}a_n + C_{ab}b_n + \cdots + C_{am}m_n = 0$$

$$\begin{aligned}
\text{2nd set: } & C_{ab}a_1 + C_{bb}b_1 + \cdots + C_{bm}m_1 = 0 \\
& C_{ab}a_2 + C_{bb}b_2 + \cdots + C_{bm}m_2 = 1 \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& C_{ab}a_n + C_{bb}b_n + \cdots + C_{bm}m_n = 0 \\
\text{nth set: } & C_{am}a_1 + C_{bm}b_1 + \cdots + C_{mm}m_1 = 0 \\
& C_{am}a_2 + C_{bm}b_2 + \cdots + C_{mm}m_2 = 0 \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& \quad \cdot \quad \quad \cdot \quad \quad \quad \cdot \quad \quad \cdot \\
& C_{am}a_n + C_{bm}b_n + \cdots + C_{mm}m_n = 1.
\end{aligned}$$

If one now lets  $R = 1/A_{1234 \dots n}$ , one can obtain the following relations for the  $c_{ij}$ 's.

From the 1st set of equations, one obtains

$$\begin{aligned}
C_{aa} &= B_{23 \dots n} R, \\
C_{ab} &= -A_{2c34 \dots n} R, \\
C_{ac} &= A_{2304 \dots n} R, \\
&\quad \cdot \\
&\quad \cdot \\
&\quad \cdot \\
C_{am} &= \pm A_{234 \dots n} R;
\end{aligned}$$

from the 2nd set, one obtains similarly,

$$\begin{aligned}
C_{bb} &= A_{1034 \dots n} R \\
C_{bc} &= -A_{1304 \dots n} R, \\
&\quad \cdot \\
&\quad \cdot \\
&\quad \cdot \\
C_{bm} &= \pm A_{134 \dots n} R;
\end{aligned}$$

and finally, from the  $n$ th set,

$$C_{mm} = A_{123 \dots (n-1)} R.$$

The values of the  $c_{ij}$ 's may then be found by obtaining the products of a number of determinants of the order  $(n - 1)$  by the reciprocal of  $A_{123 \dots n}$ , which itself may be evaluated from the relation

$$A_{123 \dots n} = A_{123 \dots (n-1)} M_n - A_{123 \dots (n-2)n} M_{n-1} + \cdots \pm A_{234 \dots n} M_1,$$

the last term being positive or negative according to the respective number of inversions of subscripts required as mentioned above. The determi-

nants which are coefficients of the  $m$ 's in the last expression are the coefficients of  $R$  in the relations for  $C_{mm}$ ,  $C_{(m-1)m}$ ,  $\dots$ ,  $C_{am}$  above.

#### APPLICATION OF THE METHOD

The application of the method will be described in connection with the solution of a set of 3 normal equations involving 3 predictors.

Normal equations:

$$Aa_1 + Bb_1 + Cc_1 = Say,$$

$$Aa_2 + Bb_2 + Cc_2 = Sby,$$

$$Aa_3 + Bb_3 + Cc_3 = Scy.$$

From the previous relations,

$$C_{aa} = B_{23}R = (b_2c_3 - b_3c_2)R,$$

$$C_{ab} = -A_{203}R = -(a_2c_3 - a_3c_2)R,$$

$$C_{ac} = A_{23}R = (a_2b_3 - a_3b_2)R,$$

$$C_{bb} = A_{103}R = (a_1c_3 - a_3c_1)R,$$

$$C_{bc} = -A_{13}R = -(a_1b_3 - a_3b_1)R,$$

$$C_{cc} = A_{12}R = (a_1b_2 - a_2b_1)R,$$

$$A_{123} = A_{12}c_3 - A_{13}c_2 + A_{23}c_1,$$

and  $R = 1/A_{123}$ .

To evaluate the  $c_{ij}$ 's, therefore, one needs to know  $A_{12}$ ,  $A_{13}$ ,  $A_{103}$ ,  $A_{23}$ ,  $A_{203}$ , and  $B_{23}$  to start with. The values of these expressions, which consist of differences between products of two terms each, as  $pq - rs$ , may be obtained from the machine without recording any intermediate figures. From these values and the original data,  $A_{123}$  and its reciprocal,  $R$ , may be evaluated, and from these, by setting  $R$  in the machine, the values of the  $c_{ij}$ 's directly. The values of  $A$ ,  $B$  and  $C$  and their standard errors may then be obtained in the usual way.

The application of the procedure outlined above to the same data from Carver used by Dwyer (2) to illustrate his method follows.

$a_1 = 1.000$	$b_1 = 0.313$	$c_1 = 0.280$	$Say = 0.495$
$a_2 = 0.313$	$b_2 = 1.000$	$c_2 = 0.652$	$Sby = 0.650$
$a_3 = 0.280$	$b_3 = 0.652$	$c_3 = 1.000$	$Scy = 0.803$
			$Syy = 1.000$

$$\begin{aligned}
A_{12} &= a_1b_2 - a_2b_1 = 0.902031 & C_{cc} &= A_{12}R = 1.758997 \\
A_{13} &= a_1b_3 - a_3b_1 = 0.564360 & C_{bc} &= -A_{13}R = -1.100525 \\
A_{103} &= a_1c_3 - a_3c_1 = 0.921600 & C_{bb} &= A_{103}R = 1.797157 \\
A_{23} &= a_2b_3 - a_3b_2 = -0.075924 & C_{ac} &= A_{23}R = -0.148055 \\
A_{203} &= a_2c_3 - a_3c_2 = 0.130440 & C_{ab} &= -A_{203}R = -0.254363 \\
B_{23} &= b_2c_3 - b_3c_2 = 0.574896 & C_{aa} &= B_{23}R = 1.121070 \\
A_{123} &= A_{12}c_3 - A_{13}c_2 + A_{23}c_1 = 0.512810 \\
R &= 1/A_{123} = 1.950040 \\
A &= C_{aa}Say + C_{ab}Sby + C_{ac}Scy = 0.270706 \\
B &= C_{ab}Say + C_{bb}Sby + C_{bc}Scy = 0.158521 \\
C &= C_{ac}Say + C_{bc}Sby + C_{cc}Scy = 0.623846
\end{aligned}$$

$$\text{Reduced } Syy = 1 - ASay - BSby - CScy = 0.262014.$$

The information contained in rows 4 to 10 inclusive, of table VII, of Dwyer's article (2), which required 37 entries (disregarding the zeros), is found in the last 12 rows of the preceding scheme, which contains but 18 entries. In addition, the preceding scheme contains the values of  $C_{ac}$ ,  $C_{ab}$ , and  $C_{bc}$ , which do not appear in said table VII, but are essential for the statistical evaluation of differences between  $A$ ,  $B$ , and  $C$ . The calculation of these values would require still 3 more entries in that table, raising the total number of entries to 40.

For the case of 4 normal equations with 4 predictors the respective numbers of entries would be 63 in the abbreviated Doolittle solution method and 40 in the proposed method. Two additional advantages of the proposed method as against Dwyer's abbreviated Doolittle solution are that the operations tend to be simpler and errors tend to be of less importance in the proposed method, since an error will not invalidate such a large proportion of the work performed subsequently to it as it does in the Doolittle technique when no check columns are included.

#### SUMMARY

A new method of solving normal equations is presented. A comparison is made of the application of the method to a multiple regression case used by Dwyer to illustrate his abbreviation of the Doolittle method. The proposed method for relatively small numbers of unknowns at least, is simpler than Dwyer's method, considered to date to be one, if not the most efficient for the purpose.

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# A METHOD OF INTERPRETING THE RESULTS OF FIELD TRIALS

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In the comparison by means of field trials of a relatively small number of agricultural practices, the complete randomized block and Latin square designs are most commonly employed by the majority of research workers. When a relatively large number of such practices are to be tested, other designs are used, such as the incomplete randomized blocks, including the lattices, and designs which make use of check plots in one form or another. The reason for this attitude is that the effects of the differences in fertility between the different plots within any given block, file or column, as the case may be, are not eliminated from the estimate of the experimental error obtained by interpreting tests performed according to the above-mentioned designs by the simple methods of statistical analysis developed for use with said types of experiments.

It occasionally happens, however, that field tests are performed in places where the fertility of the soil varies a great deal between spots relatively near to one another. Under these conditions, and especially with crops in connection with which relatively large plots must be used, the usual method of statistical analysis yields too high an estimate of error and it is impossible to determine any significant difference between the practices under trial. The possibility of the use of some alternate method of interpreting the results of such experiments might conceivably be of benefit in a number of cases. It is the purpose of this article to suggest the use of a method applicable to cases of this kind which occur when relatively small numbers of practices have been tested.

The method is based on the assumption of a different effect constant for every different pair of adjacent plots in the field when an even number of plots has been used. If an odd number of plots has been used, the previous assumption is made for all but 3 plots lying together in the field, for all three of which the same effect constant is assumed to hold. By fitting then a multiple regression equation to the results of such an experiment, it is possible to obtain an estimate of the effect constant of each of the practices tested. Mathematical statisticians certainly need no more information with respect to the way in which the method works than the one conveyed in the three preceding sentences. The rank and file of field workers, however, are not so fortunate in this respect. Due to this, the explanation of the method will be performed by following a numerical example throughout for a case where an even number of plots was used. The slight modi-

fication needed for the cases of odd numbers of plots will be dealt with further on.

DIAGRAM I  
*Field arrangement of alfalfa experiment*

50	40	30	20	10
A	H	G	F	E
30	26	30	35	44
49	39	29	19	9
C	J	I	H	G
37	36	29	30	34
48	38	28	18	8
E	B	A	J	I
59	38	22	29	33
47	37	27	17	7
I	F	E	D	C
60	42	35	24	30
46	36	26	16	6
G	D	C	B	A
62	52	45	38	33
45	35	25	15	5
D	A	J	I	H
57	33	39	40	48
44	34	24	14	4
H	F	D	C	B
43	38	43	39	49
43	33	23	13	3
E	I	H	G	F
39	36	41	45	57
42	32	22	12	2
F	C	B	A	J
43	42	47	40	52
41	31	21	11	1
J	G	F	E	D
48	46	50	52	56

Diagram I shows the field arrangement of the plots of an alfalfa experiment performed at the Agricultural Experiment Substation farm at Isabela by Messrs. L. A. Serrano and C. J. Clavell. In each case, the first number

is the plot identification number, the letter beneath it is the treatment identification letter, and the number beneath it is the observed yield in tons of green alfalfa roughage per acre obtained from 16 successive cuttings.

In table I the treatments are described, and in addition, the total and mean yields obtained with each treatment are presented.

Although in this experiment ten different treatments were tested, each one of the treatments may be expressed in terms of the amounts of nitrogen, phosphorus and potassium applied per acre. Thus, instead of trying to determine whether the observed difference in mean yields caused under the effects of any two given treatments is significant or not, one may try to determine whether the nitrogen applications have affected the alfalfa yields

TABLE I  
*Yields in tons of green alfalfa roughage per acre*

Letter	Treatments						Yields	
	Units applied			Lbs./A.				
	N	P	K	NH <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Total	Mean
A	0	0	0	0	0	0	158	31.6
B	0	1	3	0	48	216	211	42.2
C	0	1	4	0	48	288	193	38.6
D	1	1	3	36	48	216	232	46.4
E	2	1	3	72	48	216	228	45.6
F	1	1	2	36	48	144	227	45.4
G	1	1	4	36	48	288	217	43.4
H	1	0	3	36	0	216	188	37.6
I	1	2	3	36	96	216	198	39.6
J	2	2	4	72	96	288	204	40.8

significantly or not; and similarly for the other two elements. In order to determine the effects of these elements, one may assume that the yield of any plot is the additive result of a series of terms as follows:

$$N_i b_n + P_i b_p + K_i b_k + B_i = Y_i, \quad (1)$$

where  $Y_i$  is the yield of plot  $i$ ;  $B_i$  is the yield constant of the block of which plot  $i$  forms part;  $N_i$ ,  $P_i$ , and  $K_i$  are the units of nitrogen, phosphorus and potassium applied to the crop in plot  $i$ ; and  $b_n$ ,  $b_p$ , and  $b_k$  are the respective increases in yield caused by the application of each unit of nitrogen, phosphorus or potassium respectively.  $b_n$ ,  $b_p$ , and  $b_k$  are termed partial regression coefficients. The values of these coefficients are then the ones to be tested to determine whether the applications of each respective element have affected the yields significantly or not.

Thus, if one assumes block 1 to consist of plots 1 and 2, and assumes



further that the yield constant of the plots in block 1 is  $B_1$ , one may write the following equation for plot 1:

$$b_n + b_p + 3b_k + B_1 = 56, \quad (2)$$

since plot 1 received treatment  $D$ , which consisted in the application of 1 unit of nitrogen, 1 unit of phosphorus and 3 units of potassium. One may similarly write the following equation for plot 2:

$$2b_n + 2b_p + 4b_k + B_1 = 52. \quad (3)$$

In a similar way, one may write down equations for the other plots, obtaining a total of 50 equations, one for each plot. In these equations there would be the 28 unknowns  $b_n, b_p, b_k, B_1, B_2, \dots, B_{25}$ . It is impossible to determine values for these 28 unknowns which will fit the 50 equations. The next best solution, that of finding the most probable values of the constants, must be resorted to. Once these most probable values are determined, one may estimate the yield of any plot by means of equation (1) above. The difference between the actually observed yield on any given plot and the value of said yield estimated by means of equation (1) is the error of estimate of the yield of that plot. Thus, for plot (1) the error of estimate would be:

$$d_1 = 56 - b_n - b_p - 3b_k - B_1, \quad (4)$$

since the actually observed yield was 56 and the estimated yield would be the value of  $b_n + b_p + 3b_k + B_1$  when the most probable values of these constants are substituted in equation (4).

According to the principle of Least Squares, the most probable values of constants like the ones under discussion are those which render the sum of the squares of the errors of estimate a minimum. Thus, if  $d_1$  is the error of estimate of plot 1,  $d_2$  is the error of estimate of plot 2, etc., those values of the constants  $b_n, b_p, b_k, B_1, B_2, \dots, B_{25}$  which would make  $Sd^2 = d_1^2 + d_2^2 + \dots + d_{50}^2$  a minimum, would be their most probable values, and therefore, the ones to be used in equation (1) for estimating the yield of any given plot.

Now,

$$d_1^2 = (56 - b_n - b_p - 3b_k - B_1)^2. \quad (5)$$

Similarly,

$$d_2^2 = (52 - 2b_n - 2b_p - 4b_k - B_1)^2. \quad (6)$$

In a similar way one may find the values of  $d_3^2, d_4^2, \dots, d_{50}^2$ .

In applying the criteria for a minimum to the expression  $Sd^2 = d_1^2 + d_2^2 + \dots + d_{50}^2 = (56 - b_n - b_p - 3b_k - B_1)^2 + (52 - 2b_n - 2b_p -$

$4b_k - B_1)^2 + \dots + (30 - B_{25})^2$ , one obtains 28 equations, each one of which is a partial derivative of  $Sd^2$  with respect to one of the constants  $b_n, b_p, b_k, B_1, B_2, \dots, B_{25}$  equated to zero. The equation obtained on finding the partial derivative of  $Sd^2$  with respect to  $B_1$  and simplifying is

$$56 - b_n - b_p - 3b_k - B_1 + 52 - 2b_n - 2b_p - 4b_k - B_1 = 0. \quad (7)$$

From this equation one gets

$$B_1 = 54 - 3b_n/2 - 3b_p/2 - 7b_k/2. \quad (8)$$

If one now substitutes this value of  $B_1$  in equation (4) above, and simplifies, one gets

$$d_1 = 2 + b_n/2 + b_p/2 + b_k/2 = (d_1 - d_2)/2. \quad (9)$$

Substituting the value of  $B_1$  in the corresponding equation for plot 2, i.e.,

$$d_2 = 52 - 2b_n - 2b_p - 4b_k - B_1,$$

$$\text{one gets } d_2 = -2 - b_n/2 - b_p/2 - b_k/2 = -(d_1 - d_2)/2. \quad (10)$$

It will be noticed that by means of these substitutions  $B_1$  has been eliminated from the equations corresponding to the first two plots and, since these are the only two equations where  $B_1$  occurs, from the whole set of 50 equations. In a similar way all the other  $B$ 's may be eliminated from the other equations resulting finally in a system of 50 equations in but the three unknowns  $b_n, b_p$ , and  $b_k$ . In this new system of equations, the right-hand side of the equation corresponding to plot 1 will be equal to the right-hand side of that corresponding to plot 2 except that the signs are reversed. Similarly the right-hand sides of the equations corresponding to plots 3 and 4 will be equal except for the signs changed; and similarly for the remaining pairs of equations.

Now, the most probable values of the three unknowns  $b_n, b_p$ , and  $b_k$  are, as stated above, those which would make  $Sd^2$  a minimum. From equations (9) and (10) above, it follows that  $d_1 = -d_2$ , and therefore,  $d_1^2 = d_2^2$  and  $-2d_1d_2 = 2d_1^2 = 2d_2^2 = d_1^2 + d_2^2$ . Thus,  $(d_1 - d_2)^2 = d_1^2 - 2d_1d_2 + d_2^2 = 2d_1^2 + 2d_2^2$ . A similar relation may be found in the cases of the corresponding errors of estimate of the plots belonging to the other blocks. Therefore,

$$\begin{aligned} (d_1 - d_2)^2 + (d_3 - d_4)^2 + \dots + (d_{49} - d_{50})^2 &= 2d_1^2 + 2d_2^2 + 2d_3^2 + 2d_4^2 \\ &+ \dots + 2d_{49}^2 + 2d_{50}^2 = 2Sd^2. \end{aligned} \quad (11)$$

For simplicity of calculations  $Sd^2$  is calculated in the method under discussion by calculating  $2Sd^2$  by the use of the left-hand side of equation (11), and dividing by 2. The numerical values of the coefficients of the

squares and products of the unknowns which appear in  $Sd^2$  may thus be conveniently found in the example under study by arranging the calculations as they appear in table II.

TABLE II  
*Calculation of the numerical values of the coefficients of squares and products*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Difference		$d_N$	$d_P$	$d_K$	$d_Y$	$d_N^2$	$d_N d_P$	$d_N d_K$	$d_N d_Y$	$d_P^2$	$d_P d_K$	$d_P d_Y$	$d_K^2$	$d_K d_Y$	$d_Y^2$
Plots	Treatments														
1-2	D-J	-1	-1	-1	4	1	1	1	-4	1	1	-4	1	-4	16
3-4	F-B	1	0	-1	8	1	0	-1	8	0	0	0	1	-8	64
5-6	H-A	1	0	3	15	1	0	3	15	0	0	0	9	45	225
8-7	I-C	1	1	-1	3	1	1	-1	3	1	-1	3	1	-3	9
10-9	E-G	1	0	-1	10	1	0	-1	10	0	0	0	1	-10	100
11-12	E-A	2	1	3	12	4	2	6	24	1	3	12	9	36	144
13-14	G-C	1	0	0	6	1	0	0	6	0	0	0	0	0	36
15-16	I-B	1	1	0	2	1	1	0	2	1	0	2	0	0	4
18-17	J-D	1	1	1	5	1	1	1	5	1	1	5	1	5	25
20-19	F-H	0	1	-1	5	0	0	0	0	1	-1	5	1	-5	25
21-22	F-B	1	0	-1	3	1	0	-1	3	0	0	0	1	-3	9
24-23	D-H	0	1	0	2	0	0	0	0	1	0	2	0	0	4
26-25	C-J	-2	-1	0	6	4	2	0	-12	1	0	-6	0	0	36
27-28	E-A	2	1	3	13	4	2	6	26	1	3	13	9	39	169
30-29	G-I	0	-1	1	1	0	0	0	0	1	-1	-1	1	1	1
31-32	G-C	1	0	0	4	1	0	0	4	0	0	0	0	0	16
34-33	E-I	1	-1	0	2	1	-1	0	2	1	0	-2	0	0	4
36-35	D-A	1	1	3	19	1	1	3	19	1	3	19	9	57	361
37-38	F-B	1	0	-1	4	1	0	-1	4	0	0	0	1	-4	16
39-40	J-H	1	2	1	10	1	2	1	10	4	2	20	1	10	100
41-42	J-F	1	1	2	5	1	1	2	5	1	2	5	4	10	25
44-43	H-B	1	-1	0	4	1	-1	0	4	1	0	-4	0	0	16
46-45	G-D	0	0	1	5	0	0	0	0	0	0	0	1	5	25
47-48	I-E	-1	1	0	1	1	-1	0	-1	1	0	1	0	0	1
49-50	C-A	0	1	4	7	0	0	0	0	1	4	7	16	28	49
Sum.....						29	11	18	133	20	16	77	67	199	1480
Coefficients (= Sum/2)....						14.5	5.5	9	66.5	10	8	38.5	33.5	99.5	740

Columns (1), (2) and (6) have been filled from the information given in diagram I. In finding the differences corresponding to any given pair of adjacent plots, said differences have been taken in such an order as to yield a positive value of  $d_Y$  in every case. Thus, since the yield of plot 2 was smaller than the yield of plot 1, the differences are to be found by subtracting the data of plot 2 from the corresponding data of plot 1. This is indicated by writing "1 - 2" in column (1). Since plot 1 received treatment D

and plot 2 received treatment  $J$ , the corresponding entry in column (2) is  $D - J$ . The entry in column (6) is  $4 (= 56 - 52)$ . The rest of columns (1), (2), and (6) is filled in a similar way. Thus, for plots 33 and 34, the order will be  $34 - 33$  since the yield of plot 34 exceeded the yield of plot 33. In columns (2) and (6) the corresponding entries are  $E - I$  and 2, respectively.

After filling columns (1), (2), and (6); columns (3), (4), and (5) must be filled. In filling them use is made of table I. The entries corresponding to plots 1 and 2 in these columns are found as follows:  $d_N =$  units of  $N$  of treatment  $D$  minus the units of  $N$  of treatment  $J = 1 - 2 = -1$ ; and likewise,  $d_P = 1 - 2 = -1$ , and  $d_K = 3 - 4 = -1$ . For plots 33 and 34, where the corresponding difference between treatments is  $E - I$ ,  $d_N = 2 - 1 = 1$ ,  $d_P = 1 - 2 = -1$ , and  $d_K = 3 - 3 = 0$ . In a similar way, the rest of columns (3), (4), and (5) may be filled.

Once columns (1) to (6) are filled, the corresponding entries in columns (7) to (16) may be made by performing in each case the operation indicated by the heading at the top of each column. Thus, for the entries corresponding to plots 1 and 2,  $d_N^2 = d_N d_N = (-1)(-1) = 1$ ;  $d_N d_P = (-1)(-1) = 1$ ;  $d_N d_K = (-1)(-1) = 1$ ;  $d_N d_Y = (-1)(4) = -4$ ;  $d_P^2 = d_P d_P = (-1)(-1) = 1$ ;  $d_P d_K = (-1)(-1) = 1$ ;  $d_P d_Y = (-1)4 = -4$ ; etc. The entries corresponding to plots 33 and 34 are, likewise, as follows:  $d_N^2 = (1)(1) = 1$ ;  $d_N d_P = (1)(-1) = -1$ ;  $d_N d_K = (1)(0) = 0$ ;  $d_N d_Y = (1)(2) = 2$ ; etc.

Once these entries are made, it remains but to add the entries of columns (6) to (16) and to divide each sum by 2. Since

$$Sd^2 = b_n^2 Sn^2 + 2b_n b_p Snp + 2b_n b_k Snk - 2b_n Sny + b_p^2 Sp^2 + 2b_p b_k Spk - 2b_p Spy + b_k^2 Sk^2 - 2b_k Sky + Sy^2, \quad (12)$$

one can write the value of  $Sd^2$  for this case as follows:

$$Sd^2 = 14.5b_n^2 + 2(5.5)b_n b_p + 2(9)b_n b_k - 2(66.5)b_n + 10b_p^2 + 2(8)b_p b_k - 2(38.5)b_p + 33.5b_k^2 - 2(99.5)b_k + 740. \quad (13)$$

As previously stated, the most probable values of  $b_n$ ,  $b_p$ , and  $b_k$  are those which will make  $Sd^2$  minimum. Among the mathematical requirements for  $Sd^2$  to be a minimum, the requirements stated by three following equations must be fulfilled:

$$b_n Sn^2 + b_p Snp + b_k Snk = Sny, \quad (14)$$

$$b_n Snp + b_p Sp^2 + b_k Spk = Spy, \quad (15)$$

$$\text{and} \quad b_n Snk + b_p Spk + b_k Sk^2 = Sky. \quad (16)$$

Equations (14), (15), and (16) are known as "normal equation in  $b_n$ ," "normal equation in  $b_p$ ," and "normal equation in  $b_k$ ," respectively. In

the sample under study, the equations are as follows:

$$14.5b_n + 5.5b_p + 9b_k = 66.5, \quad (17)$$

$$5.5b_n + 10b_p + 8b_k = 38.5, \quad (18)$$

and 
$$9b_n + 8b_p + 33.5b_k = 99.5. \quad (19)$$

These three simultaneous linear equations in 3 unknowns may be solved by any of the well-known methods studied in the elementary courses in algebra to obtain the values of the partial regression coefficients  $b_n$ ,  $b_p$ , and  $b_k$ . Due, however, to the fact that the knowledge of the standard error of any given statistic is almost as essential as the value of the statistic itself for the proper evaluation of the significance of said statistic and of differences between it and some other statistic, Fisher's modification of Gauss' method of correlatives or indeterminate multipliers is usually resorted to in practice. Snedecor (4, p. 302) and Rider (3, p. 39) present discussions and give examples of solutions of these systems of equations by this method. Doolittle's method of solution of normal equations as discussed by Mills (2, p. 656) may be incorporated to advantage to the method of indeterminate multipliers. The author has found these methods, however, too tedious and intricate for use in practice. He suggests, therefore, the use of the following method which, for a small number of constants to be fitted, is much easier and takes considerably less time to apply than the methods previously referred to, specially if one has a printed or mimeographed form stating the calculations to be performed, so that one has to fill in merely the values asked for in said form. The suggested method of solution is as follows:

Following Fisher, (1, p. 144), the equations to be solved are:

$$C_{aa}a_1 + C_{ab}b_1 + C_{ac}C_1 = 1, \quad (20)$$

$$C_{aa}a_2 + C_{ab}b_2 + C_{ac}C_2 = 0, \quad (21)$$

$$C_{aa}a_3 + C_{ab}b_3 + C_{ac}C_3 = 0, \quad (22)$$

$$C_{ab}a_1 + C_{bb}b_1 + C_{bc}C_1 = 0, \quad (23)$$

$$C_{ab}a_2 + C_{bb}b_2 + C_{bc}C_2 = 1, \quad (24)$$

$$C_{ab}a_3 + C_{bb}b_3 + C_{bc}C_3 = 0, \quad (25)$$

$$C_{ac}a_1 + C_{bc}b_1 + C_{cc}C_1 = 0, \quad (26)$$

$$C_{ac}a_2 + C_{bc}b_2 + C_{cc}C_2 = 0, \quad (27)$$

and 
$$C_{ac}a_3 + C_{bc}b_3 + C_{cc}C_3 = 1, \quad (28)$$

where  $a_1 = Sn^2$ ,  $a_2 = b_1 = Snp$ ,  $a_3 = c_1 = Snk$ ,  $b_2 = Sp^2$ ,  $b_3 = c_2 = Spk$ , and  $c_3 = Sk^2$ .

Now, if  $A_{12}$  represents the determinant of the second order  $\begin{vmatrix} a_1 & b_1 \\ a_2 & b_2 \end{vmatrix}$ ,  $A_{123}$  means the determinant of the third order  $\begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix}$ , and, in general,  $A_{123\dots n}$  means the determinant of the  $n$ th order

$$\begin{vmatrix} a_1 & b_1 & \dots & m_1 \\ a_2 & b_2 & \dots & m_2 \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ a_n & b_n & \dots & m_n \end{vmatrix},$$

the following relations may be easily deduced from the fact that any given determinant is equal to the sum of all terms which may be formed by writing down the principal diagonal  $a_1b_2c_3 \dots m_n$  and forming all possible permutations of the subscripts; the sign of each term being positive if an even number of inversions must be performed to bring the subscripts into an ascending series and negative if said number of inversions is odd.

$$A_{12} = a_1b_2 - a_2b_1; \quad A_{123} = A_{12c_3} - A_{13c_2} + A_{23c_1},$$

$$A_{1234} = A_{123d_4} - A_{124d_3} + A_{134d_2} - A_{234d_1}, \text{ etc.}$$

If now  $A_{103} = a_1c_3 - a_3c_1$ ;  $A_{203} = a_2c_3 - a_3c_2$  and  $B_{23} = b_2c_3 - b_3c_2$ , the values of  $C_{aa}$ ,  $C_{ab}$ , and  $C_{ac}$  may be determined from equations (20), (21), and (22) above to be as follows:

$$C_{aa} = B_{23}A_{123}^{-1}; \quad C_{ab} = -A_{203}A_{122}^{-1}; \quad \text{and} \quad C_{ac} = A_{23}A_{123}^{-1}.$$

From equations (23), (24), and (25), the values of  $C_{bb}$  and  $C_{bc}$  come out to be as follows:

$$C_{bb} = A_{103}A_{123}^{-1}; \quad \text{and} \quad C_{bc} = -A_{13}A_{123}^{-1}.$$

Finally, from equations (26), (27), and (28),

$$C_{cc} = A_{12}A_{123}^{-1}.$$

The number of operations required to evaluate these statistics is not large and does not take too long, specially if, as already stated, one has some form indicating the operations and has to fill only the numbers in it. Such a form, already filled, appears as Table III.

In this table,  $A$  corresponds to  $b_n$ ,  $B$  corresponds to  $b_p$ , and  $C$  corresponds

to  $b_k$  which are the unknowns whose values were to be determined. These values of  $b_n$ ,  $b_p$ , and  $b_k$  may be checked by substituting them in equations (17), (18), and (19) above.

TABLE III  
*Solution of simultaneous linear equations*

$Aa_1 + Bb_1 + Cc_1 = Say$ $Aa_2 + Bb_2 + Cc_2 = Sby$ $Aa_3 + Bb_3 + Cc_3 = Scy$			
$a_1 = 14.5$	$b_1 = 5.5$	$c_1 = 9$	$Say = 66.5$
$a_2 = 5.5$	$b_2 = 10$	$c_2 = 8$	$Sby = 38.5$
$a_3 = 9$	$b_3 = 8$	$c_3 = 33.5$	$Scy = 99.5$
$a_1b_2 = 145$	$a_2b_1 = 30.25$	$a_3b_1 = 49.5$	$b_2c_3 = 335$
$a_1b_3 = 116$	$a_2b_3 = 44$	$a_3b_2 = 90$	$b_3c_2 = 64$
$a_1c_3 = 485.75$	$a_2c_3 = 184.25$	$a_3c_1 = 81$	
		$a_3c_2 = 72$	
$A_{12} = a_1b_2 - a_2b_1 = 114.75$		$A_{12}c_3 = 3844.125$	
$A_{13} = a_1b_3 - a_3b_1 = 66.50$		$-A_{13}c_2 = -532$	
$A_{103} = a_1c_3 - a_3c_1 = 404.75$		$A_{23}c_1 = -414$	
$A_{23} = a_2b_3 - a_3b_2 = -46$		$A_{123} = 2898.125$	
$A_{203} = a_2c_3 - a_3c_2 = 112.25$		$A_{123}^{-1} = 0.000345051$	
$B_{23} = b_2c_3 - b_3c_2 = 271$			
$C_{aa} = B_{23}A_{123}^{-1} = 0.0935088$		$C_{bb} = A_{103}A_{123}^{-1} = 0.139659$	
$C_{ab} = -A_{203}A_{123}^{-1} = -0.0387320$		$C_{bc} = -A_{13}A_{123}^{-1} = -0.0229459$	
$C_{ac} = A_{23}A_{123}^{-1} = -0.0158723$		$C_{cc} = A_{12}A_{123}^{-1} = 0.0395946$	
Checks:			
$C_{aa}a_1 = 1.35588$	$C_{ab}a_2 = -0.213026$	$C_{ac}a_3 = -0.142851$	
$C_{ab}b_1 = -0.213026$	$C_{bb}b_2 = 1.39659$	$C_{bc}b_3 = -0.183567$	
$C_{ac}c_1 = -0.142851$	$C_{bc}c_2 = -0.183567$	$C_{cc}c_3 = 1.32642$	
<u>1.0000</u>	<u>1.0000</u>	<u>1.0000</u>	
$C_{aa}Say = 6.218335$	$C_{ab}Say = -2.575678$	$C_{ac}Say = -1.055508$	
$C_{ab}Sby = -1.491182$	$C_{bb}Sby = 5.376872$	$C_{bc}Sby = -0.883417$	
$C_{ac}Scy = -1.579294$	$C_{bc}Scy = -2.283117$	$C_{cc}Scy = 3.939663$	
<u>A = 3.147859</u>	<u>B = 0.518077</u>	<u>C = 2.000738</u>	

The significance of these coefficients of regression may be checked by calculating their standard errors and using the "t-test." The total sum of squared deviations corrected for variations in fertility from block to block was found in table II to be 740, subject to 25 degrees of freedom, since from the total original number of 49 degrees of freedom, 24 ( $= 25 - 1$ ) belong to the block statistics assumed to exist. The reduction in this

sum of squares caused by fitting the statistics  $b_n$ ,  $b_p$ , and  $b_k$  is found, as usual, by means of the relation

$$Sy^2 = b_n Sn^2 + b_p Sp^2 + b_k Sk^2 = 209.33 + 19.95 + 199.07 = 428.35, \quad (29)$$

corresponding to 3 degrees of freedom, since it corresponds to the three statistics so fitted. The reduced sum of squared deviations is, therefore,  $Sd^2 = S(Y - Y')^2 = 740 - 428.35 = 311.65$ , corresponding to 22df.

The estimate of the variance,  $V$ , is, therefore,  $V = 311.65/22 = 14.1659$ . The variance of  $b_n$  is then  $C_{aa}V = (0.0935088)14.1659 = 1.3246$ . The standard error of  $b_n$  is  $S.E._{b_n} = (1.3246)^{1/2} = 1.151$ , and the corresponding value of  $t = 3.1479/1.151 = 2.73$ , which is significant at the 5% point; since the value of  $t$  at the 5% for 22df. is 2.074.

TABLE IV.  
*Analysis of the total sum of squared deviations*

Source of the deviations	Degrees of freedom	Sum of squared deviations	Variance estimate	F values		
				Experi-mental	5%	1%
Total .....	49	4,651				
Blocks .....	4	802				
Treatments...	9	922	102.4	1.26	2.15	2.94
Error...	36	2,927	81.31			

Similarly,  $V_{b_p} = 1.9784$ ,  $S.E._{b_p} = 1.407$ , and  $t_{b_p} = 0.37$ , which is not significant. For  $b_k$ , the corresponding figures are:  $V_{b_k} = 0.5609$ ,  $S.E._{b_k} = 0.7489$ , and  $t_{b_k} = 2.67$ , which is also significant at the 5% point.

The performed analysis indicates that the crop responded significantly to the applications of nitrogen and potash, whereas it did not do so with respect to those of phosphorus.

The usual analysis of variance of the yield data of the experiment under study yielded table IV, the first block consisting of plots 1 to 10, the second block of plots 11 to 20, etc.

The usual hypothesis thus fails to show any significant effect of the treatments on the yields. However, since in the previous analysis but 3 statistics were fitted to explain the effects of the fertilizer applications whereas in this analysis 9 such statistics were fitted, a new analysis was made in an attempt to explain the sum of squares due to treatments in this last analysis by the use of only 3 statistics. Such an attempt indicated that of the sum of squares due to treatments, 922, a total of 334 could be explained by fitting the 3 statistics, there remaining 588 to be assigned to interactions of one sort or another. If this remainder is pooled with the sum of squares due to error, 2,927, a total of 3,515 is obtained, subject to



$36 + 6 = 42\text{df}$ . The new estimate of the error variance would be, therefore, 83.69, of the same order of magnitude as the value formerly obtained of 81.31. On testing the fitted statistics, whose values came out to be:  $b_n = 2.167$ ,  $b_p = 0.529$  and  $b_k = 1.188$ , it is found that none of them is significant.

On comparing the variance estimate obtained by the last method of analysis, 83.69, with that obtained by the proposed method, 14.1659, one obtains a relative precision of 5.9078, ( $= 83.69/14.1659$ ), in favor of the 2-plot blocks hypothesis as against the usual hypothesis, in this case the 10-plot blocks hypothesis. There has been, in this case, therefore, an increase in precision of 491 per cent due to the change in hypothesis.

The results of this experiment might have been also interpreted by assuming 5-plot incomplete blocks consisting of either the plots receiving treatments A, C, E, G and I or treatments B, D, F, H, and J. The results of such an analysis appear in table V.

TABLE V  
*Analysis of the total sum of squared deviations*

Source of the deviations	Degrees of freedom	Sum of squared deviations	Variance estimate	F values		
				Experimental	5%	1%
Total....	49	4,651				
Blocks.....	9	2,317				
Treatments.....	8	830	103.75	2.21	2.25	3.12
Error.....	32	1,504	47.00			

The observed differences between the treatment means are again not significant, although there has been an increase in precision of  $81.31/47 - 100\% = 73\%$ , by the use of the smaller 5-plot blocks.

In case that an experiment consists of an odd number of plots, either by design or accident, the contribution of all but 3 plots to the numerical coefficients of the squares and products which appear in  $Sd^2$  will be calculated as described above in the case of table II, by finding the respective differences between the adjacent plots, finding all possible squares and products of these differences, adding these squares and products, and dividing each of these sums by 2.

The contribution of the 3 plots left out in the previous calculations may be computed as follows: The set of differences corresponding to the first of these plots will be formed by subtracting the sum of the corresponding figures of the second and third plot from twice the corresponding figures of the first plot. The set of differences corresponding to the second plot will be likewise formed by subtracting the sum of the corresponding figures of the first and third plot from twice the corresponding figures of the

second plot. In a similar way are found the differences corresponding to the third plot.

Again the sums of all possible squares and products of these sets of differences are found, but in this case the sums must be divided by 9 in order to determine the contributions of these last 3 plots to the numerical coefficients. The sum of the contributions of both sets of plots will give the required numerical coefficients of the squares and products which appear in  $Sd^2$ .

To illustrate the way of finding the contribution of these last 3 plots in such an analysis, one may assume that plots 48, 49, and 50 of diagram I are the remaining 3 plots in the case of an experiment with an uneven number of plots. The value of  $d_r$  for plot 48 would be found as follows: Since plot 48 received treatment  $E$ , with 2 units of nitrogen; plot 49 received treatment  $C$ , with no nitrogen; and plot 50 received treatment  $A$ , with no nitrogen, the respective difference,  $d_N$ , would be, therefore,  $2(2) - 0 - 0 = 4$ . The corresponding  $d_P$  would be  $2(1) - 1 - 0 = 1$ ; the corresponding  $d_K$  would be  $2(3) - 4 - 0 = 2$ ; and the corresponding  $d_r$  would be  $2(59) - 37 - 30 = 118 - 67 = 51$ . In a similar way, the differences corresponding to plots 49 and 50 are found.

In the above discussion, the question of the significance of the difference between any pair of statistics fitted to data of the kind mentioned has been dispensed with. In the cases where several varieties or field practices have been tested, the interest centers, not on whether a given variety or practice produces a significant departure of the measured effect from the mean effect of all the varieties or practices tested, but on whether the given variety or practice causes a significantly greater or smaller effect than some other variety or practice included in the test. In such cases, the number of statistics fitted to the data is one less than the number of treatments under study. Thus, if, say, varieties  $A, B, C, D$ , and  $E$  were tested in one of such experiments, statistics for  $A, B, C$ , and  $D$  only would be fitted, it being understood that the statistic for  $E$  is equal to the negative sum of the statistics corresponding to the other four varieties, i.e.,  $E = -(A + B + C + D)$ . The corresponding  $C_{ei}$ 's would be found by means of the relations:

$$C_{ea} = -(C_{aa} + C_{ab} + C_{ac} + C_{ad}), \quad (30)$$

$$C_{eb} = -(C_{ab} + C_{bb} + C_{bc} + C_{bd}), \quad (31)$$

$$C_{ec} = -(C_{ac} + C_{bc} + C_{cc} + C_{cd}), \quad (32)$$

$$C_{ed} = -(C_{ad} + C_{bd} + C_{cd} + C_{dd}), \quad (33)$$

$$\text{and} \quad C_{ee} = -(C_{ae} + C_{be} + C_{ce} + C_{de}). \quad (34)$$

The variance estimate of a difference between any two such statistics, say  $A$  and  $B$ , would be found by means of the relation  $(C_{aa} - 2C_{ab} + C_{bb})V$ ,

from which the corresponding values of the standard error and  $t$  would be found accordingly.

It now remains to present a resumé of the results obtained to date in the use of the 2-plot blocks method in the interpretation of the results of field trials. It may be stated that, in general, and in full accord with the theoretical foundations of the method, it is most useful as compared with

TABLE VI

*Comparison of efficiency of the 2-plot blocks method with the usual analysis of "variance" method of interpreting the results of complete randomized blocks experiments*

Crop	Nature of test	Factor studied	Coefficient of variability obtained by usual analysis of "variance" method	Increase in efficiency due to the use of the 2-plot blocks method
			%	%
Corn, 1st crop.....	Fertilizer	Yield	11.04	13.15
Corn, 2nd crop....		"	17.13	-6.43
Corn, 3rd crop.....		"	9.97	-11.00
Alfalfa, 16 cuttings.....		" unlimed	21.93	490.81
" " " "		" limed	7.13	32.60
Beans.....		"	16.79	27.86
Sweet potato, 1st crop		"	43.71	248.75
" " " 2nd "		"	26.92	51.29
Cucumbers, 1932-33..		"	18.38	11.31
Cucumbers, 1933-34...		"	18.20	109.32
" " " 1935-36..	Varietal	"	27.67	74.88
" " " 1936-37....		"	21.02	75.15
" " " 1941-42...		"	25.25	24.81
Cotton.....		"	16.27	-7.61
Sugar cane, Aguirre....		Yield of cane	17.27	53.48
" " " 1942-43		"	"	"
" " " ".....		Tons sugar/A	17.31	56.93
" " " ".....		Sugar % cane	4.62	-13.18
Sugar cane, Río Piedras		"	"	"
1941-42.....		Tons sugar/A	27.60	190.16

the usual method of analysis in cases where soil heterogeneity affects markedly the effect under study, the more so the more variable the soil. In table VI, the results of comparisons of the 2-plot blocks method with the usual method of analysis are presented. In every case the comparisons between the two methods have been made by using the same number of statistics to explain the effects of the treatments.

As seen above, in by far the majority of the cases, the 2-plot blocks method has been more precise than the complete randomized blocks

method. The method, however, takes more time and work to apply to the results of trials not specially designed for its application. By using some sort of balanced design, however, the work of calculation of the results of experiments by means of the proposed method may be considerably shortened.

Thus, in testing 6 treatments, each of the treatments might be included with each of the other ones an equal number of times in the 2-plot blocks. This would require replicating each treatment either 5 times or some multiple of 5. In general, this would require  $m(n - 1)$  replications where  $n$  is the number of treatments tested and  $m$  is any positive integer. Such a layout for 6 treatments replicated 5 times could be something as illustrated in diagram II.

DIAGRAM II

*Layout for a plot distribution to test 6 treatments (A, B, C, D, E, and F) each replicated 5 times*

AB	CD	EF
EC	FA	BD
FD	BC	AE
CA	DE	FB
BE	CF	DA

The advantage of such a balanced design would be that all  $C_{ii}$ 's would be equal, i.e.,  $C_{aa} = C_{bb} = C_{cc} = C_{dd} = C_{ee} = C_{ff}$ , and, similarly, all  $C_{ij}$ 's would be equal, i.e.,  $C_{ab} = C_{ac} = \dots = C_{af}$ . Therefore, the same variance estimate would be used to test each effect constant and, likewise, there would be but one variance estimate to test the difference between any two such effect constants. More information relative to the possibilities of the use of these balanced designs will be given in another article to be published shortly after this one in this same Journal. In said article, the manuscript of which has been already prepared, the modified calculational technique is discussed and a numerical example is presented.

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# A NEW METHOD OF PERFORMING FIELD TRIALS

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## INTRODUCTION

In a recent article (1) in which the author discussed a new method of interpreting the results of field trials, he presented evidence tending to show that the assumption of a different effect constant for every pair of adjacent plots of a randomized block field trial led to a greater precision in the reduction of the corresponding data than the assumption of a different effect constant for every different complete block of the experiment. In said article he presented a method which might be used in this connection in order to reduce the calculational work to a minimum for the interpretation of such experiments by use of the proposed new assumption. The work involved in the application of said method to the results of randomized block experiments already performed, however, is greater than the work required for the application of the usual methods of statistical analysis in regard to which said experiments have been designed. On the other hand, the increase in precision obtained by the application of the suggested method seems to the author to warrant fully the increase in such calculational work.

As it was also mentioned in the article referred to, it is possible to reduce the number of operations required for the application of that method to the interpretation of field trials by designing such field trials so as to make use of the proposed method. The purpose of this article is to suggest designs for possible use in this connection as well as to discuss the procedure to be followed in the interpretation of such specially designed experiments.

## REQUIREMENTS TO BE FULFILLED BY FIELD TRIALS

Field trials must fulfill certain requirements in order that the application of the proposed method to the interpretation of their results be most economical in time and effort. These requirements are as follows: 1—As many different groups of two treatments each must be made as is possible. 2—The experimental field must be divided into blocks of 2-adjacent plots each, so that the topographies of the two plots in any given block will be as nearly alike as possible. 3—Each group of two treatments must be assigned at random to be tested in one of the 2-plot blocks. The plot of the respective 2-plot block in which one or the other of the two treatments of the group assigned to it will be tested must be also decided at random. 4—The same number of replications of each group of treatments must be used. That is,

if  $n$  treatments are to be tried, there should be  $m(n - 1)$  replications, where  $m$  is any positive integer. Thus, if 6 treatments are to be compared, either 5 or 10 or 15, etc. replications of the treatments are required.

#### EXAMPLES OF DESIGNS OF POSSIBLE USE

In order to give illustrations of the possibilities of this type of layout, two examples will be given of possible different designs for a trial in which 5 treatments are repeated 4 times each.

Where 5 treatments, say  $A, B, C, D$ , and  $E$ , are to be repeated 4 times each, the experiment should include 10 groupings as follows:  $AB, AC, AD, AE, BC, BD, BE, CD, CE$ , and  $DE$ . The 20-plot experimental field is divided into ten 2-plot blocks, and the above treatment pairs are

DIAGRAM I  
*Possible layouts of plots*

(a)	(b)
AB BC	AB EC D
EC EA	ED CB A
AD BE	AE BD C
ED AC	CA EB D
DB CD	

assigned at random to be tested in one or another of the 10 blocks, there being also randomization of the order of the treatments to be tested within each block. A possible geographical distribution might be as indicated by Diagram I (a).

Another possible arrangement, which lends itself to possible interpretation by either the 2-plot block method or by the ordinary method of analysis for a 4-randomized-block trial, is shown in Diagram I (b).

#### THEORY OF THE PROCEDURE OF CALCULATION

As indicated in the article (1) previously referred to, the regression equation to be used in explaining the results of such an experiment would be

$$Y_i = B_i + T_i, \quad (1)$$

where  $Y_i$  is the observed effect, say yield, of plot  $i$ ,  $B_i$  is the effect constant of the block of which plot  $i$  forms part, and  $T_i$  is the effect constant of the treatment tested in plot  $i$ . After writing down the respective equation

for each of the plots used, the problem becomes one of finding the most probable values of the  $B_i$ 's and the  $T_i$ 's, and the standard errors of the latter for comparison with one another. It was suggested in said article that a convenient way to do this would be one in which the differences between the corresponding figures of the paired plots were found and then, following the usual methods of regression analysis, determining the most probable values of the  $T_i$ 's and their standard errors.

Now, if the experiment has been carried out in such a way that every treatment has been thus paired with every other treatment *once*, it will be found that by the application of the above procedure, the value of each of the  $C_{ii}$ 's will come out to be  $2(n-1)/n^2$  and the value of each of the  $C_{ij}$ 's ( $i \neq j$ ) will come out to be  $-2/n^2$  where  $n$  is the number of treatments tested.

By knowing then  $S_{ay}$ ,  $S_{by}$ ,  $S_{cy}$ ,  $S_{dy}$ ,  $S_{ey}$ , and  $S_{yy}$ , and by the proper use of the  $C_{ii}$ 's and  $C_{ij}$ 's, already known, the values of the treatment effect constants, i.e.,  $A$ ,  $B$ ,  $C$ ,  $D$ , and  $E$ , may be found together with their standard errors. One may thus test the significance between the estimated difference in effects between any two of the treatments tested.

The theory of the procedure outlined in the last paragraph is so well known to those interested in it, that it does not merit further consideration. It may be worthwhile, however, to derive the relations  $C_{ii} = 2(n-1)/n^2$  and  $C_{ij}(i \neq j) = -2/n^2$ . This will be done now.

Let it be assumed that  $n$  treatments have been tested in a trial of the kind under consideration. Let the effects of the different treatments above or below the mean effect of all treatments be designated by  $A$ ,  $B$ ,  $C$ ,  $\dots$ ,  $N$ , where  $N$  is the effect of  $n$ th treatment. The effect equations of the type

$$Y_i = B_i + T_i$$

may then be written for each plot.

Since any given treatment, say  $A$ , has been replicated  $n-1$  times, there will be  $n-1$  such equations in which the treatment effect  $A$  appears. Also, on account of this, there will be  $n-1$  equations formed by finding the differences between the equations corresponding to the treatment  $A$  plots and those corresponding to the other plots paired to said treatment  $A$  plots. These equations might be written as:

$$\begin{aligned} \pm A \mp B &= d_{\pm A \mp B} \\ \pm A \mp C &= d_{\pm A \mp C} \\ \pm A \mp D &= d_{\pm A \mp D} \\ &\cdot \quad \cdot \quad \cdot \\ &\cdot \quad \cdot \quad \cdot \\ &\cdot \quad \cdot \quad \cdot \\ \pm A \mp (N-1) &= d_{\pm A \mp (N-1)} \\ \pm A \mp N &= d_{\pm A \mp N} \end{aligned}$$

where the  $d$ 's stand for the respective differences between the effects noticed in the  $A$  plots as against the effects noticed in the plots paired with the  $A$  plots. Since, however,  $A, B, C, \dots$ , and  $N$  have been assumed to be the effects of the treatments above or below the mean effect of all the treatments,

$$A + B + C + \dots + N = 0,$$

or

$$N = -(A + B + C + \dots + (N - 1)).$$

The last of the above equations, therefore, should appear rather as  $\pm 2A \pm B \pm C \pm \dots \pm (N - 1) = d_{\pm A \mp N}$ , due to the relation for  $N$  previously pointed out. Further, the effect constant  $A$  would also appear in all the equations corresponding to comparisons between plots receiving treatment  $N$  and anyone of the other treatments.

In other words,  $A$  appears with a coefficient of  $\pm 1$  in the  $n - 2$  equations corresponding to treatment  $A$  comparisons with treatments  $B, C, D, \dots$ ,  $N - 1$ , and also in the  $n - 2$  equations corresponding to the comparisons of treatments  $B, C, D, \dots$ , and  $N - 1$  with treatment  $N$ ; and furthermore, with a coefficient of  $\pm 2$ , in the equation corresponding to its comparison with treatment  $N$ . That is,  $A$  will appear with a coefficient of  $\pm 1$  in  $2n - 4$  equations and with a coefficient of  $\pm 2$  in one equation. This is true also of each of the other treatment effect constants.

Therefore, the sum of the *squares* of the  $A$  coefficients will be  $(2n - 4) + 4 = 2n$ . This is true also of the sum of the squares of the coefficients of any other treatment effect constant. But since, as was demonstrated in the former article, on finding the sums of squares and products of the differences between the coefficients of the treatment effect constants in the original equations, one would be finding really  $2Sa^2$ ,  $2Sab$ ,  $\dots$ ,  $2Sa(n - 1)$  instead of  $Sa^2$ ,  $Sab$ ,  $\dots$ ,  $Sa(n - 1)$  which are the figures sought, the above value of  $2n$  must be divided by 2, so that the value of  $Sa^2 = Sb^2 = \dots = S(n - 1)^2 = n$ .

Now, in the equation corresponding to the comparison of  $A$  with  $B$ , one of the treatment effect constants is  $+1$  and the other  $-1$ , so that their product is  $-1$ . In the equation corresponding to the comparison of  $A$  with  $N$ , the coefficient of  $A$  will be  $\pm 2$  and that of  $B$  will be  $\pm 1$ , so that the products of these coefficients will be  $+2$ . This is true also of the equation corresponding to the comparison of  $B$  with  $N$ , where the coefficient of  $B$  is  $\pm 2$  and the coefficient of  $A$  is  $\pm 1$ . In each of the  $n - 3$  equations corresponding to the comparisons of each of the other treatments with  $N$ , the coefficients of both  $A$  and  $B$  will be  $\pm 1$ , so that their product in each case will be  $\pm 1$ . Therefore, the sum of the products of the coefficients of



$A$  and  $B$  in these equations will be  $-1 + 2 + 2 + (n - 3) = n$ . Again, this must be divided by 2 in order to get the proper value of  $Sab$ . Thus,  $Sab = Sac = \dots = Sa(n - 1) = Sbc = \dots = S(n - 2)(n - 1) = n/2$ .

The normal equations will be, therefore,

$$\begin{array}{rcl} An + Bn/2 + Cn/2 + \dots + (N - 1)n/2 & = & Say, \\ An/2 + Bn + Cn/2 + \dots + (N - 1)n/2 & = & Sby, \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \cdot & & \cdot \\ An/2 + Bn/2 + Cn/2 + \dots + (N - 1)n & = & S(n - 1)y, \end{array}$$

giving rise to the  $(n - 1)$  columned determinant

$$D_n = \underbrace{\begin{vmatrix} n & n/2 & n/2 & \dots & n/2 \\ n/2 & n & n/2 & \dots & n/2 \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ \cdot & \cdot & \cdot & & \cdot \\ n/2 & n/2 & n/2 & \dots & n \end{vmatrix}}_{(n - 1) \text{ columns}}$$

from which the values of  $C_{aa}$ ,  $C_{ab}$ ,  $\dots$ ,  $C_{(n-1)(n-1)}$  may be evaluated as follows.

By subtracting the last row in  $D_n$  from each of the other rows, adding the first to the last column in the expression thus obtained, expanding the expression thus obtained by minors along the first row, and by continuing such additions of the first to the last column and expansions by minors along the first row in the resulting expressions, one obtains finally

$$D_n = \left(\frac{n}{2}\right)^{n-3} \begin{vmatrix} n & 0 \\ \frac{n}{2} & n + (n - 2) \frac{n}{2} \end{vmatrix} = \left(\frac{n}{2}\right)^{n-2} \left[ n + (n - 2) \frac{n}{2} \right] = n \left(\frac{n}{2}\right)^{n-1}.$$

Now,  $C_{aa} = N_{aa}/D_n$ , where

$$N_{aa} = \underbrace{\begin{vmatrix} n & n/2 & \dots & n/2 \\ n/2 & n & \dots & n/2 \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ n/2 & n/2 & \dots & n \end{vmatrix}}_{(n - 2) \text{ columns}}$$

The evaluation of  $N_{aa}$ , following a procedure identical to that used in evaluating  $D_n$ , yields the expression

$$N_{aa} = \left(\frac{n}{2}\right)^{n-1} \begin{vmatrix} n/2 & 0 \\ n/2 & n + (n-3)\frac{n}{2} \end{vmatrix} \\ = \left(\frac{n}{2}\right)^{n-1} \left[ n + (n-3)\frac{n}{2} \right] = (n-1) \left(\frac{n}{2}\right)^{n-2}.$$

$C_{aa}$  is, therefore, equal to  $2(n-1)/n^2$ . On finding the corresponding expressions for the other treatments, it is found that  $N_{aa} = N_{bb} = \dots = N_{(n-1)(n-1)}$  and, therefore,  $C_{aa} = C_{bb} = \dots = C_{(n-1)(n-1)} = 2(n-1)/n^2$ .

Now,  $C_{ab} = N_{ab}/D_n$ , where

$$-N_{ab} = \begin{vmatrix} n/2 & n/2 & \dots & n/2 \\ n/2 & n & \dots & n/2 \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ n/2 & n/2 & & n \end{vmatrix} \\ \underbrace{\hspace{10em}}_{(n-2) \text{ columns}}$$

This determinant may be evaluated by subtracting the first row from each of the other ones and expanding the resulting expression by minors along the first column and continuing with expansions of the same kind successively with the resulting expressions, obtaining finally  $\left(\frac{n}{2}\right)^{n-2}$ . Thus,

$N_{ab} = -\left(\frac{n}{2}\right)^{n-2}$ , and  $C_{ab}$  comes out to be  $-2/n^2$ . The determinants from which  $N_{ac}$ ,  $N_{ad}$ ,  $\dots$ , and  $N_{(n-2)(n-1)}$  are to be evaluated will differ in every case from that used to evaluate  $N_{ab}$ , but by proper inversions of rows and columns they can be brought to coincide with the above one, so that  $N_{ac} = N_{ad} = \dots = N_{(n-2)(n-1)}$  and therefore,  $C'_{ab} = C'_{ac} = \dots = C_{(n-2)(n-1)} = -2/n^2$ .

In experiments of this nature where each treatment is repeated  $2(n-1)$  times,  $N_{ii}$  is  $(n-1) \left(\frac{n}{2}\right)^{n-2} 2^{n-2} = (n-1)(n)^{n-2}$ ,  $N_{ij} = -\left(\frac{n}{2}\right)^{n-2} 2^{n-2} = -(n)^{n-2}$ , and  $D_n = n \left(\frac{n}{2}\right)^{n-1} (2)^{n-1} = n(n)^{n-1}$ . Therefore,  $C_{ii} = \frac{(n-1)n^{n-2}}{n(n)^{n-1}} = \frac{n-1}{n^2}$ , and  $C_{ij} = \frac{-(n)^{n-2}}{n(n)^{n-1}} = \frac{-1}{n^2}$ . That is, as the sums of squares and products double, the values of the  $C_{ii}$ 's and  $C_{ij}$ 's are divided

by 2. If the treatments are repeated  $3(n - 1)$  times,  $C_{ii} = \frac{1}{3} \left( \frac{2(n - 1)}{n^2} \right)$  and  $C_{ij} = \frac{1}{3} \left( \frac{-2}{n^2} \right)$ , and similarly for other multiples of  $n - 1$  replications.

In the above discussion no reference has been made to  $C_{nn}$  and to the  $C_{in}$ 's, that is, the values corresponding to the last variety. Now  $C_{nn} = C_{aa} = C_{bb} = \dots = C_{(n-1)(n-1)}$  and  $C_{in} = C_{ab} = C_{ac} = \dots = C_{(n-2)(n-1)}$ . This may be proved as follows:  $N = -(A + B + C + \dots + (N - 1))$ , and therefore, for  $n - 1$  replications,  $C_{an} = -(C_{aa} + C_{ab} + C_{ac} + \dots + C_{a(n-1)}) = -C_{aa} - (n - 2)C_{ab} = \frac{-2(n - 1)}{n^2} - (n - 2) \left( \frac{-2}{n^2} \right) = \frac{-2}{n^2} = C_{ab} = C_{ac} = \dots = C_{(n-2)(n-1)}$ . This holds also for  $C_{bn}$ ,  $C_{cn}$ ,  $\dots$ ,  $C_{(n-1)n}$ . Also,  $C_{nn} = -(C_{an} + C_{bn} + \dots + C_{(n-1)n}) = -(n - 1) \left( \frac{-2}{n^2} \right) = 2(n - 1)/n^2 = C_{aa}$ , etc. The above relations hold also for other multiples of  $(n - 1)$  replications.

### Numerical example

Diagram II shows the geographical distribution of the plots, together with the treatments and yields per plot, in cwts. per acre, of a fertilizer experiment with cotton performed by Messrs. A. Riollano and J. Pastor Rodríguez in cooperation with the author, on Coto clay at Mantilla Farm, Isabela.

Column (1) of table I shows one way in which the plots may be paired so that one may employ the procedure of calculation discussed above. As will be noticed, the paired plots lie side by side in all but two cases: where plot 56 has been paired to plot 46, and where plot 35 has been paired to plot 47. In these two cases the topography of the field in the section where these plots were located suggested that they should be paired. Columns (2) to (8) show the coefficients of the treatment constants corresponding to each of the paired plots. The constant of treatment  $H$  has been expressed in terms of the constants of the other seven treatments according to the relation  $H = -(A + B + C + D + E + F + G)$ . Column (9) shows the respective differences between the yields of the paired plots, taken in the order indicated in column (1). Though unnecessary, the plots of each pair were paired so that these differences in yields were positive. The numbers in the central portions of columns (10) to (16) are the products of the entries in columns (2) to (8) by those of column (9), as expressed at the tops of the respective columns. The first of the 2 rows at the bottom of columns (10) to (16) consists of the sums of the

entries in the body of each of the respective columns. The entries of the second row at the bottom of these columns are each one-half of the entries of the first row and indicate the values of *Say*, *Sby*,  $\dots$ , *Sgy*, according

DIAGRAM II

*Geographical distribution of plots of fertilizer experiment with cotton*

52 D 5.0	53 A 6.1	54 B 6.3	55 F 7.6	56 C 9.5		
48 H 4.2	49 C 5.0	50 H 6.6	51 A 5.5			
42 F 8.6	43 G 6.7	44 E 5.9	45 G 5.2	46 B 8.0	47 D 7.8	
36 E 7.3	37 H 3.4	38 D 5.5	39 F 7.1	40 E 8.3	41 H 3.5	
29 C 6.4	30 G 6.4	31 B 7.3	32 C 6.2	33 A 4.0	34 G 8.5	35 B 8.4
22 D 7.2	23 A 5.3	24 F 6.6	25 G 8.3	26 D 6.5	27 E 8.5	28 C 8.7
15 E 6.8	16 F 7.2	17 H 3.8	18 B 8.1	19 H 5.5	20 A 5.6	21 F 8.4
8 C 4.6	9 D 7.6	10 A 4.0	11 G 7.7	12 F 8.7	13 B 9.9	14 E 7.0
1 B 6.5	2 E 5.2	3 G 7.0	4 D 8.1	5 A 5.5	6 C 7.2	7 H 6.0

to whether the heading of their respective columns are *AY*, *BY*,  $\dots$ , *GY*. The theoretical reason for this procedure of finding *Say*, *Sby*,  $\dots$ , *Sgy* has been given elsewhere (1).

Now, in this experiment each of the 8 treatments has been paired once

TABLE I  
Calculation of *Say*, *Sby*, ..., *Sgy*

(1) Plots	(2) A	(3) B	(4) C	(5) D	(6) E	(7) F	(8) G	(9) Y	(10) AY	(11) BY	(12) CY	(13) DY	(14) EY	(15) FY	(16) GY	(17) YY
1-2		1			-1		-1	1.3		1.3		1.1	-1.3		-1.1	1.69
4-3			1					1.1								1.21
6-5	-1							1.7	-1.7		1.7					2.89
14-7	1	1	1	1	2	1	1	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.00
15-8			-1		1			2.2			-2.2		2.2			4.84
9-16				1		-1	1	0.4				0.4		-0.4		0.16
10-17	2	1	1	1	1	1	1	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.04
18-11		1					-1	0.4		0.4						0.16
12-19	1	1	1	1	1	2	1	3.2	3.2	3.2	3.2	3.2	3.2	6.4	3.2	10.24
13-20	-1	1				-1		4.3	-4.3	4.3						18.49
28-21			1					0.3			0.3			-0.3		0.09
27-26				-1	1			2.0				-2.0	2.0			4.00
22-29			-1	1				0.8			-0.8	0.8				0.64
30-23	-1						1	1.1	-1.1						1.1	1.21
31-24		1				-1		0.7		0.7				-0.7		0.49
25-32			-1				1	2.1			-2.1				2.1	4.41
40-33	-1				1			4.3	-4.3				4.3			18.49
34-41	1	1	1	1	1	1	2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	25.00
35-47		1		-1				0.6		0.6		-0.6				0.36
42-36					-1	1		1.3					-1.3	1.3		1.69
38-37	1	1	1	2	1	1	1	2.1	2.1	2.1	2.1	4.2	2.1	2.1	2.1	4.41
43-44					-1		1	0.8					-0.8		0.8	0.64
39-45						1	-1	1.9						1.9	-1.9	3.61
56-46		-1	1					1.5		-1.5						2.25
49-48	1	1	2	1	1	1	1	0.8	0.8	0.8	1.6	0.8	0.8	0.8	0.8	0.64
53-52	1			-1				1.1	1.1			-1.1				1.21
50-54	-1	-2	-1	-1	-1	-1	-1	0.3	-0.3	-0.6	-0.3	-0.3	-0.3	-0.3	-0.3	0.09
55-51	-1					1		2.1	-2.1					2.1		4.41
									-0.2	17.5	11.2	12.7	18.1	19.1	17.6	114.36
									-0.1	8.75	5.6	6.35	9.05	9.55	8.8	57.18

with each of the other treatments, and as mentioned in the section on the theory of the procedure of calculation,

$$C_{aa} = C_{bb} = \dots = C_{gg} = C_{hh} = \frac{1}{2} 2 (8 - 1)/8^2 = 14/64 = 7/32,$$

and

$$C_{ab} = C_{ac} = \dots = C_{bc} = C_{bd} = \dots = C_{gh} = -2/8^2 = -2/64 = -1/32.$$

Knowing, then, the  $C_{ii}$ 's and the  $C_{ij}$ 's, together with  $Say$ ,  $Sby$ ,  $\dots$ ,  $Sgy$ , one can calculate the yield constants corresponding to each treatment and test the significance of differences between these constants.

Thus,

$$\begin{aligned} A = b_A &= C_{aa}Say + C_{ab}Sby + C_{ac}Scy + C_{ad}Sdy \\ &\quad + C_{ae}Sey + C_{af}Sfy + C_{ag}Sgy \\ &= (7Say - Sby - Scy - Sdy - Sey - Sfy - Sgy)/32, \\ &= (8Say - (Say + Sby + Scy + Sdy + Sey + Sfy + Sgy))/32 \\ &= (-0.8 - 48.00)/32 = -48.8/32 = -1.525. \\ B = b_B &= (8Sby - 48.00)/32 = (70.00 - 48.00)/32 = 22/32 = 0.6875 \\ C = b_C &= (8Scy - 48.00)/32 = (44.80 - 48.00)/32 \\ &= -3.2/32 = -0.1000 \\ D = b_D &= (8Sdy - 48.00)/32 = (50.80 - 48.00)/32 = 2.8/32 = 0.0875 \\ E = b_E &= (8Sey - 48.00)/32 = (72.40 - 48.00)/32 = 24.4/32 = 0.7625 \\ F = b_F &= (8Sfy - 48.00)/32 = (76.40 - 48.00)/32 = 28.4/32 = 0.8875 \\ G = b_G &= (8Sgy - 48.00)/32 = (70.40 - 48.00)/32 = 22.4/32 = 0.7000 \\ H &= -(A + B + C + D + E + F + G) = -1.5000. \end{aligned}$$

To make comparisons between these statistics, one must calculate their standard errors. The sum of the squared deviations of the yields corrected for differences in fertility between the different 2-plot blocks is found by dividing the sum of the squares of the entries in column (9) of table I by 2. The squares of the corresponding entries constitute the body of column (17) of table I, their sum is the next to the last item in that column, and the required sum of squared deviations corrected for differences in fertility between the 2-plot blocks is one-half the next to the last item in column (17), that is, 57.18. This sum of squared deviations is subject to 28 degrees of freedom, since 28 degrees of freedom have been lost: one  $df$  corresponding to the mean of all the yields and 27  $df$  due to the fitting of the 27 different block constants necessary to express the relations between the fertilities of the 28 2-plot blocks.

The reduction of the sum of squared deviations due to the fitting of the treatment constants to the yield data is, according to the usual relation,  $Sy'y' = ASay + BSby + \dots + GSgy = (-1.525)(-0.1) + 0.6875(8.75) + \dots + 0.7000(8.80) = 27.70$ , subject to 7  $df$ .

The reduced sum of squared deviations is, therefore,  $S(Y - Y')^2 = S_{yy} - S_y'y' = 57.18 - 27.70 = 29.48$ , subject to  $28 - 7 = 21$  *df*. The estimate of the reduced variance,  $V'$ , is then  $29.48/21 = 1.4037$ .

The reduction in the sum of squared deviations due to the use of the 7 treatment constants may be tested by means of the *F*-test as follows:  $F = 27.70/7(1.4037) = 27.70/9.8259 = 2.82$ . Since the value of *F* at the 5% point, for 7 and 21 *df* is 2.49, the reduction in the sum of squared deviations due to the fitting of the treatment constants is a significant one.

The total sum of squared deviations of the yields of this experiment is 134.29, and as found above, the sum of squared deviations of the yields corrected for differences in fertility between blocks is 57.18. The fitting of the block constants led therefore to a reduction of  $134.29 - 57.18 = 77.11$  in the sum of squared deviations. These relations are indicated in table II.

TABLE II

*Analysis of the total sum of squared deviations following the 2-plot block method*

Source of the deviations	Degrees of freedom	Sum of squared deviations	Variance estimate	<i>F</i> values		
				Experimental	5%	1%
Total....	55	134.29				
Blocks . . . .	27	77.11				
Treatments	7	29.48	4.21	2.82	2.49	3.65
Error	21	27.70	1.4037			

In testing by means of the *t*-test any difference between the fitted treatment constants, the variance of any such difference would be found by multiplying  $V'$  by the corresponding factor of the nature of  $C_{ii} - 2C_{ij} + C_{jj}$ . Since, in the case of this experiment all the  $C_{ii}$ 's are equal and all the  $C_{ij}$ 's are equal, the variance of the difference between any two treatment constants is  $(7/32 - 2(-1/32) + 7/32) 1.4037 = 0.70185$ . The standard error of any such difference is, therefore,  $(0.70185)^{1/2} = 0.8378$ , and any such difference, to be significant at the 5% point, must exceed  $2.080(0.8378) = 1.74$  cwt. cotton per acre.

The treatments compared in this test are described in table III. The corrected mean yield of any given treatment is found by adding algebraically the respective treatment yield constant as found above to the mean yield of all plots, 6.64.

Since treatments *E*, *F*, and *G* differed only with respect to the rate of the  $K_2O$  applications, the comparisons of their corrected mean yields or, what is equivalent, of their treatment yields constants, will furnish information on the effect of the  $K_2O$  applications on the cotton yields. Such

comparisons indicate that the effect of the  $K_2O$  applications on the cotton yields was not significant since the largest difference between any two of said constants was only 0.1875, whereas such a difference would have to exceed 1.74 cwts./A. to be significant at the 5% point.

Treatments *C*, *D*, and *G* varied only with respect to the  $P_2O_5$  applications. In this case also, none of the differences between the yield constants were significant although they were of a larger magnitude than in the case of the  $K_2O$  applications.

A similar comparison of the yield constants of treatments *A*, *B*, and *G* indicates that there was a significant increase in yield, caused by the application of at least 100 lbs.  $NH_3$  per acre, since the difference between the yield constants of treatments *A* and *B* was 2.22 cwts. cotton per acre, and the corresponding difference between the yield constants of treatments

TABLE III  
*Description of treatments and corrected mean yields*

Treatment letter	Lbs. of nutrient applied per acre			Treatment yield constant	Corrected mean yield (cwts./acre)
	$NH_3$	$P_2O_5$	$K_2O$		
A	0	200	200	-1.5250	5.11
B	100	200	200	0.6875	7.33
C	200	0	200	-0.1000	6.54
D	200	100	200	0.0875	6.73
E	200	200	0	0.7625	7.40
F	200	200	100	0.8875	7.53
G	200	200	200	0.7000	7.34
H	0	0	0	-1.5000	5.14

*A* and *G* was 2.23 cwts. cotton per acre, both differences being significant at the 5% point and nearly so at the 1% point. The difference between the yield constants of treatments *B* and *G* was not significant indicating that the second 100 lbs.  $NH_3$  applied per acre did not affect significantly the yields already obtained with the first 100 lbs.  $NH_3$  applied per acre.

The yield constant of treatment *H* was almost equal to that of treatment *A*, corroborating the conclusions drawn above that nitrogen was the only one of the three elements tested which affected the yields significantly.

This experiment might have also been interpreted by using only 3 constants to explain the variations in yield caused by the various treatments as described in the article (1) previously referred to. Such a study was made yielding the same results previously obtained, except that the partial regression coefficient of the yields on the nitrogen applications came out to be significant at the 1% point.



## SUMMARY

A new method of performing field experiments with relatively small numbers of treatments is described. The requirement to be fulfilled by the layouts of such field tests is specified and examples of possible designs for a 5-treatment experiment are illustrated. The theory of the procedure of calculation is discussed and a numerical example of said calculations is furnished in connection with the interpretation of a fertilizer experiment performed with cotton.

## REFERENCE

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## THE COTTONS OF PUERTO RICO

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The Cottons of the Antillean region belong to the New World cultivated group of species of *Gossypium*. They are perennial shrubs or small trees, and they all bear spinnable lint. On them was based the first expansion of cotton growing in the New World when the mechanical inventions of the Industrial Revolution created a demand greater than the cultivations of the Old World could meet. Though Puerto Rico does not seem to have developed cotton growing to the same extent as the British Islands, which had the advantage of direct access to English markets, there is evidence that it shared to some extent in the seed exchanges whereby the cottons of the indigenous inhabitants of the West Indian Islands were supplemented by commercially desirable types from South and Central America.

The rise of the southern United States as a great cotton producing area and the attacks of cotton pests combined to reduce the profit from cotton production below that obtainable from sugar, and except in one or two isolated areas such as the Grenadine islands, the cotton crop vanished completely from West Indian agriculture. Nevertheless, cotton lint has its domestic uses, and both leaves and roots are employed in household remedies. Many people, therefore, plant a shrub in the house-yard, or encourage a volunteer seedling. In Trinidad (Hutchinson, 1943b), this appears to be the plant's chief means of survival. In Jamaica and the Leeward Islands some types belonging to the species *G. hirsutum* are established as components of the vegetation of xerophytic scrub lands, and others occur spontaneously in badly drained wooded areas on the banks of streams (e.g. Antigua "fringing forests"). Ecological specialisation between species is also evident, the South American *G. barbadense* being more frequent in moist areas and the Caribbean *G. hirsutum* var. *marie-galante* in dried zones. Thus the perennial cottons, abandoned as an export crop, have become established both as commensal plants and also as members of natural plant associations.

At the end of the nineteenth century, with the decline of the sugar industry, cotton again became a commercial crop in the West Indies. The

old perennials however, could not compete with the more recent, high quality, annual Sea Island varieties. Indeed, where Sea Island cotton became a major crop, the old types were a menace to the new, since they provided a source of contamination by mixing and hybridisation, and a host reservoir for pests such as blister mite, stainers, and later pink bollworm. They were therefore exterminated, and in such islands as St. Vincent and Montserrat the clean-up has been so thorough that no perennial cotton has been seen for years.

Indigenous types, and those on which the cultivations of the eighteenth and nineteenth centuries were based, can only be studied in islands where Sea Island cotton has never been established, or is only of secondary interest. The cottons of Jamaica and Trinidad have already been discussed (Hutchinson, 1943a, 1943b) and the available information on early cultivations in the West Indies as a whole has been summarised by Stephens (1944). The interest of the Puerto Rican cottons is two fold. Firstly, studies of geographical distribution (Hutchinson, in press) have shown that the Greater Antilles form the northern limit of *G. hirsutum* var. *marie-galante* and the southern limit of *G. hirsutum* var. *punctatum*. The latter does not occur in Jamaica, but has been recorded from Haiti, Cuba, and (as herbarium material) Puerto Rico. Secondly, it is of some importance to see how general is the ecological distinction between *G. barbadense* and *G. hirsutum* var. *marie-galante*, and whether there is a similar distinction between these two and *G. hirsutum* var. *punctatum*. A visit to Puerto Rico in January 1944 provided an opportunity to study the distribution and ecology of its perennial cottons, and the information collected is here reported on.

It is necessary first to consider briefly the classification of the New World cultivated species. A detailed classification is given elsewhere (Hutchinson, in press). It is sufficient to say here that the results of cytological and genetic research have been used to identify those natural groups which are separated by real genetic barriers. Only these have been given species rank. Morphologically and genetically distinct sub groups which, when brought together, interbreed freely giving genetically balanced, fully fertile progeny in  $F_2$  and later generations, are regarded as of varietal rank only. Geographical and ecological races that cannot be separated by good morphological criteria are regarded as below the limit of taxonomic differentiation, as also are the manifold variations to be found within an unselected, interbreeding crop population.

The application of these principles has resulted in reducing the species of New World cultivated cottons to two, *G. barbadense* L., the South American cotton, and *G. hirsutum* L., the Central American cotton. Both

are extremely variable. In *G. barbadense*, varietal distinctions are rather ill-defined. Besides the type, two varieties are separated, chiefly on capsule characters, var. *braziliense*, the form with very large capsules and usually kidney seeds from eastern South America, and var. *darwinii*, the wild form with small capsules from the Galapagos islands. In *G. hirsutum*, on the other hand, varietal distinctions are much more strongly marked, though since they are primarily physiological and ecological, they offer some difficulty to the herbarium taxonomist. The typical form is the early annual Upland cotton of the Mexican plateau and the Cotton Belt of the United States. Upland cottons have not been reported from Puerto Rico. In lowland Central America and round the coasts and islands of the Gulf of Mexico is to be found the shrubby perennial var. *punctatum*. It is an early fruiting type, often producing two crops a year. It branches from near the base of the main stem forming, when old, a thicket of thin flexible branches without a definite main stem or trunk. Round the coasts of the Caribbean, south on the American mainland to Brazil, and north through the Antilles is to be found var. *marie-galante*, the Marie-galante cotton of the Grenadines and the Moco of Brazil. The *marie-galante* cottons are large shrubs or small trees, almost always having a definite main stem or trunk. They are photoperiodic, flowering being confined to the months with short days, so they only produce one crop a year. These species and varieties may be recognised as follows:

- a. Staminal column long; anther filaments short, spreading, all about the same length; anthers closely packed; capsule surface usually pitted, with black oil glands in the pits—b.
  - b. Capsules usually less than 6 cm. long, broadest near the base; seeds free—c.
    - c. Capsules small, usually about 3 cm. long, finely pitted.
      - G. barbadense* var. *darwinii*
    - cc. Capsules large, well filled, usually 4–6 cm. long, usually coarsely pitted.
      - G. barbadense*
  - bb. Capsules very long, usually more than 6 cm. long, broadest near the middle, tapering to the base, coarsely pitted; seeds usually joined.
    - G. barbadense* var. *braziliense*
- aa. Staminal column short; anther filaments longer above than below. the upper ones usually ascending; anthers openly spaced; capsule surface smooth—d.
  - d. Bracteole teeth 6–14 (usually about 10); anthers rather sparsely and irregularly arranged, capsules rounded or ovate—e.
    - e. Annual sub-shrubs with few large leaves, and few vegetative branches.
      - G. hirsutum*
    - ee. Perennial, much branched shrubs with small leaves.
      - G. hirsutum* var. *punctatum*
  - dd. Bracteole teeth 3–11 (usually about 6); anthers very many, and regularly arranged on long filaments; capsules tapering; perennial shrubs or small trees, usually having a definite trunk.
    - G. hirsutum* var. *marie-galante*

The cottons of Puerto Rico have been discussed by Watt (1907), Britton and Wilson (1924), and Stahl (1936). Under *G. barbadense* should be included Watt's *G. barbadense*, *G. vitifolium* and *G. microcarpum*. Britton and Wilson's *G. barbadense*, *G. peruvianum* and *G. microcarpum*, and Stahl's *G. barbadense*. *G. barbadense* also includes the cultivated annual Sea Island cottons. *G. barbadense* var. *braziliense* is the *G. braziliense* of Watt, Britton and Wilson and Stahl.

The classification of the cottons now included under *G. hirsutum* vars. *punctatum* and *marie-galante* has been very confused, since the characteristic habit features by which they are best distinguished are not visible on herbarium material. In var. *punctatum* are included *G. purpurascens* and *G. racemosum*, both of which appear to have been described from Puerto Rican plants. Chevalier (1939) has stated that the type specimen of *G. racemosum* Poir was collected in Puerto Rico in 1796 by André-Pierre Le Dru, and he suggested that the seeds which were planted in the Museum garden in Paris and gave rise to the material on which *G. purpurascens* was based, were collected by Le Dru on the same expedition. Watt rightly regarded *G. racemosum* as a synonym of *G. purpurascens*, but he failed to recognise the group now assigned to *G. hirsutum* var. *marie-galante*. Hence, he assigned most of the Puerto Rican material he examined to *G. purpurascens*. It is difficult to determine from their descriptions whether either Britton and Wilson, or Stahl had a clear conception of the distinction between the two groups. It seems likely that Britton and Wilson's *G. hirsutum* and Stahl's *G. racemosum* are *G. hirsutum* var. *punctatum* and their *G. purpurascens* is *G. hirsutum* var. *marie-galante*, but is not possible to decide with certainty.

*G. janiphaefolium* Bello, which has only been collected once or twice, is referred to by both Britton and Wilson, and Stahl. Mr. R. A. Silow of this Station has examined material in the Gray herbarium, and states that it is a laciniated leaved form of *G. hirsutum* either var. *marie-galante* or var. *punctatum*. Laciniated leaved races of the former are common in Jamaica, and of the latter in Mexico.

In interpreting observations on the present distribution of cottons in Puerto Rico it is necessary to bear in mind the effect of recent attempts to exterminate "wild" cottons in the interests of the Sea Island cotton crop. In most areas the perennials have merely been reduced in number, but in the northwestern cotton area they have been practically eliminated, and the types that formerly occurred there can only be inferred from those found in neighbouring areas, and from ecological factors influencing the distribution of cottons in other parts of the island.

Perennial cottons are common in the coastal plains, uncommon in the inland hilly country, and apparently absent from higher elevations.

None were seen on the road over El Yunque between Mameyes and the Rio Blanco. On three routes across the island (Rio Piedras-Caguas-Juncos-Humacao-Maunabo; Caguas-Cayey-Salinas; Arecibo-Lares-Yauco) only one cotton shrub was seen beyond the limits of the southern plains and their foothills near Salinas and Yauco. This one plant was a *G. barbadense* type, and was found between Caguas and Cayey.

Along the north coast practically all the perennial cottons seen were forms of *G. barbadense*. Plants were recorded in house-yards at intervals from Naguabo on the east coast to Arecibo on the north coast. One plant was noted in a house-yard in Mayaguez and another in Cabo Rojo. There can be no doubt that they were formerly to be found also in the northwest district from Arecibo to Mayaguez. No kidney seeded var. *braziliense* cottons were seen, though they have been recorded by earlier collectors. Considerable variability was observed in characters of no taxonomic importance. Yellow and pale flowers, large and small petal spot, and fuzzy and tufted seed were seen, and the staple length as measured on combed halos varied from 35 to 46 mm. In the plaza in San Juan there is a very large cotton plant with large fuzzy seeds, and very coarse, short sparse lint. It is near *G. barbadense*, but presents some unusual features. It is included here pending observation in culture. *G. barbadense* was only seen in house-yards, and never recorded as occurring spontaneously in uncultivated areas. Most of the plants seen bore ripe seed cotton, but only the early capsules had burst.

The commonest cotton in Puerto Rico is *G. hirsutum* var. *marie-galante*. This is the "wild cotton" of house-yards all through the south coast region from Guayama to Lajas and Mayaguez. On the north coast it is rare, but plants were seen by the road side in dry country near Fajardo, and in house-yards in the environs of San Juan and near Vega Alta. Capsules were just bursting and plants with no ripe seed cotton were still common. Though generally found in house-yards, it was not infrequently seen in poorly drained areas on the margins of streams in the southern sugar cane belt.

Variability in *marie-galante* was very low, in marked contrast to the wide range of types found in Jamaica (Hutchinson 1943a). Most plants were glabrous or nearly so. Hairy types occur occasionally. The seeds of those examined were all clean of fuzz except for a small green tuft at one or both ends. Fuzzy seeded plants have been recorded by Dr. L. F. Martorell, but appear to be rare. The lint is of fair quality, not very copious, and was fairly uniform (about 40 mm. long) in the samples examined.

*G. hirsutum* var. *punctatum* was only collected in the south of the island, and appears to be comparatively rare. A considerable colony of plants



was seen growing spontaneously on poorly drained land adjacent to the airfield at Central Mercedita, near Ponce, and another colony on sandy wasteland close to the landing ground in the Guanica Insular Forest. All plants were early fruiting and when examined were covered with ripe capsules. A striking feature of both colonies was their variability. At Central Mercedita light brown and white lint, fuzzy and semi-fuzzy seed were noted, and in the Guanica Forest there were tufted and naked seeds and also a type (found by Dr. L. F. Martorell) with naked, lintless kidneyed seeds. The lint of the linted types was fairly uniform, rather more copious, but probably rather inferior to that of var. *marie-galante*, with a halo length varying from 32–40 mm. In the two collections a greater range of variation was recorded than was observed among all the var. *marie-galante* plants examined. The discovery of a lintless, kidney seeded type is of particular interest in view of Watt's (1907) comment that "Todaro was in error when he placed this plant (*G. racemosum* Poir) under his subsection *Synspermia* and figured the seeds as naked and kidneyed." Chevalier's (1939) plate shows that Poiret's type was linted, but Todaro evidently saw specimens of the form found by Martorell. The lintless kidney seeded type must have persisted in Puerto Rico for a long time.

In discussing the justification for regarding *G. racemosum* as a synonym of *G. purpurascens*, Watt referred to specimens collected in 1885 by Paul Sintenis and labelled "Salinus de Cabo Rojo in sylvis litoralibus." He commented that "the specimens collected by Sintenis are distinctly peculiar. The leaf stalks are pale pink and the flowers very small and numerous. It is spoken of as found in forests along the seashore, from which circumstance it may have been wild." He suggested that this might be Poiret's *G. racemosum* but from Chevalier's description and plate it is clear that Poiret's type was the common form of var. *punctatum*. The Salinas near the Cabo Rojo lighthouse are protected from the sea by a sand ridge, classified in the Puerto Rico Soil Survey as Palm Beach Sand. It is too dry and windswept to support a forest, but it carries an open plant community composed of shrubs, grass, and cactus. It is well known as a locality in which wild cottons occurs, and although much has been eliminated in the cotton eradication campaign, we had no difficulty in finding bushes among the cactus and scrub. At Dr. Martorell's suggestion we also looked for it at the Salinas de Guanica, which is the only other locality on the south coast where Palm Beach Sand is found, and we found the same cotton in very similar ecological conditions. This type is known as "algodón brujo," and is evidently truly wild. In vegetative characters it is as described by Watt. At the time of our visit it was fruiting freely, and many of the capsules had burst. The capsules are small, smooth, and almost round, containing small seeds with hard seed coats. The seeds bear a thick brown fuzz and a rather sparse and irregular coat of

brown lint. The Cabo Rojo sample gave a combed halo length of 27 mm. and the Guanica samples 40 mm.

"Algodón brujo" is indistinguishable from the wild cotton hitherto known as *G. taitense* Parl., *G. taitense* has been regarded as endemic in Polynesia, but there has been considerable difficulty in determining the limits of its distribution, since it cannot be distinguished with certainty from *G. hirsutum* var. *punctatum*. Watt assigned to it specimens from New Caledonia, the Philippines, Rodriguez and Madagascar, and similar types are known from Haiti, and (as herbarium material) from various localities in Central America. Watt (1907) acknowledged the difficulty of separating *G. taitense* from *G. purpurascens*, which is here included in *G. hirsutum* var. *punctatum*. Harland (1939) also remarked that it seemed too closely related to the *punctatum* cottons to have been isolated in the Pacific for a long period of time. Recent genetic work at the Cotton Research Station, Trinidad, has confirmed Harland's belief that the wild cotton of Fiji is genetically very close to the *punctatum* cottons of the Gulf of Mexico, and the discovery of a cotton indistinguishable from the Fijian form growing wild in Puerto Rico completes the case for including *G. taitense* in *G. hirsutum* var. *punctatum*.

The distribution of *G. barbadense* and *G. hirsutum* var. *marie-galante* is in conformity with their distribution in the other islands studied. *G. barbadense* is confined to the more mesophytic areas with high rainfall, and *G. hirsutum* var. *marie-galante* is common in dry, rather xerophytic areas, though it is also occasionally found where *G. barbadense* predominates. *G. hirsutum* var. *punctatum* appears to be adapted to even more xerophytic conditions than var. *marie-galante*. The Central Mercedita colony of the typical form was growing in the same sort of situation as var. *marie-galante* occupies, but the Guánica Forest colony was in a more xerophytic habitat than any in which var. *marie-galante* was found. "Algodon brujo" is a highly xerophytic race adapted to drier conditions than would be tolerated by any other Puerto Rican cotton.

All cottons are light loving plants. Seedlings are only established successfully in the open, and mature plants are rarely found where there is overhead shade. In the West Indies suitable conditions are to be found only in cultivated land and the immediate vicinity of houses, and in the natural vegetation of such areas as are too dry for the development of a complete plant cover. *G. barbadense*, which has only recently been introduced from South America, has only been recorded in house-yards, and commercial cultivations. The *marie-galante* cottons are most common in house-yards also, but they are to be found in abandoned lands that are swampy during part of the year, and in pastures. The fact that they are spontaneous in such localities cannot be regarded as evidence that they are indigenous, since all these areas owe their present status to the activi-

ties of man, and if left alone would revert to closed forest in which cottons would not survive.

Both forms of var. *punctatum* have been found in situations where they owe nothing to man. That "algodón brujo" is truly wild is beyond doubt, and the typical var. *punctatum* found in the Guanica Insular Forest occurred in a natural plant association. In both cases the plant communities are open, low scrub associations, where considerable patches of bare ground occur, offering good opportunities for the establishment of light loving seedlings. In Puerto Rico, therefore, there is every reason to believe that *G. hirsutum* var. *punctatum* is the native cotton, both *G. hirsutum* var. *marie-galante* and *G. barbadense* being either directly or indirectly dependent upon man for survival. This conclusion is in sharp contrast to that reached from a similar study of the cottons of Jamaica (Hutchinson 1943a) where the only var. *punctatum* seen was a plot planted with imported seed, and where var. *marie-galante* was found growing spontaneously in xerophytic scrub land.

Studies of *marie-galante* x *punctatum* hybrids have shown that there is no genetic barrier between them. If they were both indigenous in the Greater Antilles, and had spread thence to other areas, one would expect to find considerable intergradation, at least at the margins of their ecological ranges. The absence of intermediates, and the specialisation of the photoperiodic var. *marie-galante* to tropical regions indicates that *marie-galante* is a comparatively recent introduction, and the Greater Antilles may be regarded as on the periphery and not at the centre of its distribution. The variability of the *marie-galante* cottons of Jamaica must then be ascribed to the introduction of a wide range of types during the great development of cotton growing in the latter part of the eighteenth century. (See Stephens 1944.) The absence of var. *punctatum* from Jamaica and the Lesser Antilles shows that the Greater Antilles are on the periphery of the distribution of this variety also. In both varieties, the cultivated and house-yard cottons that are found in association with mankind have a wider geographical distribution and are found in a wider range of ecological situations than the wild races of xerophytic scrub lands, and it is evident that man has had a very large part in the dissemination of cotton through the Antillean region.

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## IRON AND MANGANESE IN RELATION TO PLANT GROWTH AND ITS IMPORTANCE IN PUERTO RICO

BY E. F. HOPKINS, VÍCTOR PAGÁN AND F. J. RAMÍREZ SILVA

In recent years attention has been focused on minor element deficiencies. Many such cases having great agricultural importance have been pointed out and remedies for their prevention have been put into practice. The matter of toxicity of the minor elements has not been emphasized to a great extent from the standpoint of practical plant culture except for certain limited cases. Further, the possibility of balance or antagonism between the minor elements themselves has been the subject of still less investigation. It is the purpose of this paper to point out that toxicity of manganese to plant growth occurs in Puerto Rico, and to attempt to make clear the mechanism by which iron antidotes this toxicity.

The toxicity of manganese to pineapple plants in Hawaii as reported by Kelley (20, 21, 22) and by Johnson (15, 16, 17, 19) might be cited as such a particular instance of toxicity just referred to. This results from the peculiarities of the soil there which develops under cultivation a high amount of soluble manganese. In fact, Gile (6, 7) in investigating chlorosis of pineapple plants on calcareous coastal plain soils in Puerto Rico found no excessive manganese and considered pineapple chlorosis on the Island to be of a different nature. In other words, it was caused by a high alkalinity bringing about a precipitation of iron and with it an iron deficiency. This may be true of these calcareous soils. Nevertheless it will be shown later in this paper that in the principal pineapple growing areas in Puerto Rico manganese toxicity chlorosis occurs on acid soils, and this chlorosis may be prevented by raising the pH with calcium carbonate.

It is of particular interest that while in Hawaii, Johnson (15, 16) found that spraying the plants with an iron sulphate solution counteracted the effect of manganese present in the soil, Gile (6) found that iron sulphate added to the soil had no effect and that while spraying the plants with this chemical would prevent chlorosis, the spraying had to be repeated every few months to keep the plants green. He concluded he was dealing with a different condition from that in Hawaii and that iron sprays were of no practical value.

The question as to whether the cause of this chlorosis on calcareous soils in Puerto Rico is fundamentally different from the other will be discussed later. However, it is now clear that in the principal pineapple growing area there exists at the present time a condition identical to that in Hawaii, i.e., high manganese and low iron on acid soils.

PRELIMINARY EXPERIMENTAL WORK WITH SOILS<sup>1</sup> AND PLANTS  
AND DISCUSSION OF THE PROBLEM

To show that the same condition exists in Puerto Rico as in Hawaii it is sufficient merely to point out the following facts resulting from our experiments and observations: 1—chemical analysis of certain soils revealed them to have a high amount of manganese soluble in distilled water varying from 20 to over 130 ppm of manganese and no detectable water soluble iron; 2—pineapple plants grown on these soils without receiving iron sprays show extreme chlorosis characteristic of manganese toxicity; 3—practical growers have found that iron sulphate spray is essential and



FIG. 1. Bean plants growing in soil from pineapple field containing a high concentration of soluble manganese. A—Check, with no additions. B—With calcium carbonate to pH 6.2. C—With calcium carbonate to pH 6.2 plus humate iron.

this is now a common practice; 4—the common bean when grown on these soils shows severe chlorosis of the first trifoliate leaves in from 6 to 10 days from planting and no further growth of the plant occurs; 5—when the manganese is immobilized by adjusting the pH of the soil to about 6.2 with calcium carbonate, chlorosis is prevented and if, in addition, iron in a soluble form (as humate iron) is used, normal growth of beans results (see figure 1); 6—even when chlorosis in pineapple plants is corrected by iron sulphate sprays, certain abnormalities occur that are undoubtedly due to manganese toxicity: there is a reduction in the size of the plant,

<sup>1</sup> The pineapple soils referred to here were obtained from the vicinity of Arecibo and Manatí. The soil type was determined by Dr. J. A. Bonnet of the Soils Department, to be "Bayamón Sandy Clay Loam."

the leaves are narrow and tend to develop more red pigment than normal; 7—plants of the variety "Red Spanish" grown from slips obtained from Cuba and which probably have a higher iron reserve are, in the first generation, much larger and greener and appear to be a different variety from those grown from Puerto Rican slips; 8—after about two generations

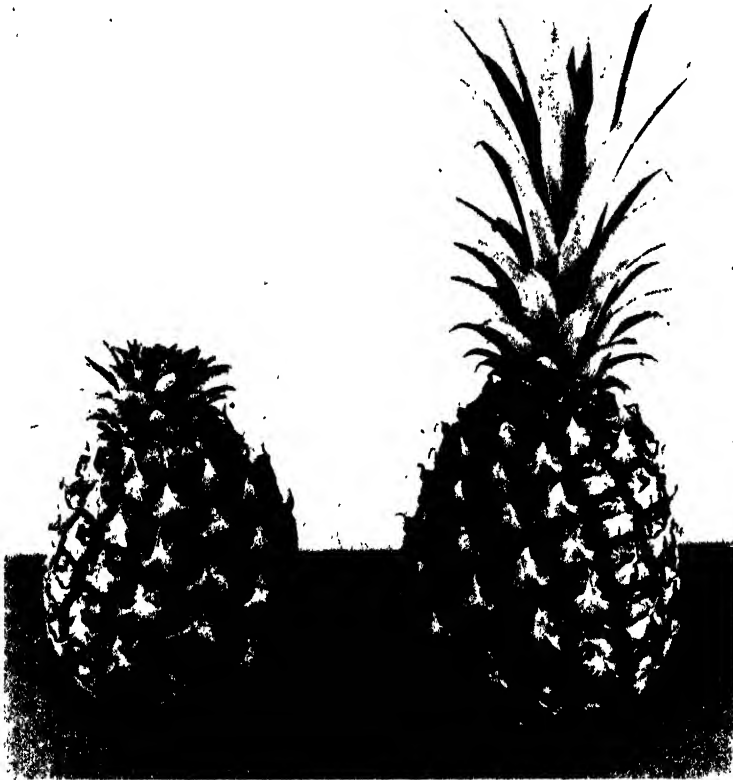


FIG. 2. Pineapple fruits showing—left—the "short top" symptoms resulting from high content of soluble manganese in the soil at high light intensity. Right, a normal fruit.

in Puerto Rico plants originally from Cuban slips revert to the type described above; 9—many fruits produced on these soils are affected with "short top"<sup>2</sup> (figure 2) which appears to be brought about by high soluble

<sup>2</sup> This was first called to our attention by Mr. John Raymer of the Palo Blanco Fruit Company, Arecibo, Puerto Rico, and has since been observed on fruit from other plantations.

manganese in combination with high light intensity; 10—ash analyses of pineapple fruits and other plants from these areas show such very high amounts of manganese that the carbon free ash when removed from the muffle furnace has the characteristic blue-green color due to a large content of manganese (figure 3).<sup>3</sup>

To what extent this soil condition exists in the West Indies, Central and South America it would be impossible to say without an extensive survey but it would appear to be not uncommon. Several Puerto Rican soils other than those used for pineapple culture were tested by the authors and found to have high amounts of water soluble manganese. Ash analyses of banana seed pieces from Guatemala made some years ago by the senior

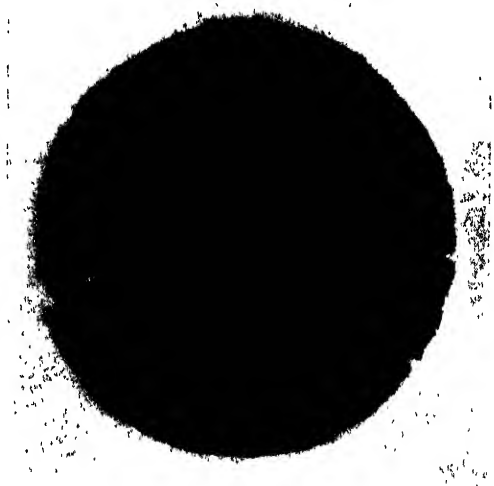


FIG. 3. Carbon free ash of pineapple fruit produced on soil with high content of soluble manganese. The "dark" color of the ash in the photograph is a blue-green color characteristic of plant ash with high manganese content.

author revealed high amounts of manganese. In continental United States a similar condition has been reported by Funchess (3). He found that certain soils in Alabama, when fertilized with either dried blood or ammonium sulphate, developed a high amount of soluble manganese very toxic to plants. One soil from Pennsylvania investigated by him showed

<sup>3</sup> Analyses of 4 fruits from iron sprayed plants and their tops (crown leaves) showed on the oven dry basis:

Fruit	Iron	75 to 200 ppm	Ave. 141 ppm Fe
	Manganese	175 to 520 ppm	Ave. 300 ppm Mn
Tops	Iron	100 to 178 ppm	Ave. 129 ppm Fe
	Manganese	250 to 1495 ppm	Ave. 871 ppm Mn

the same phenomenon. Toxic concentrations of soluble or "available" manganese in soils have also been reported from Kentucky (27, 1, 31), Connecticut (14), Indiana (2), New South Wales (8), Sweden (34), India (9), and probably occur in many other places.

This brings up the matter of fertilizer practice in relation to the problem. It was pointed out by one pineapple grower in Puerto Rico that he obtained much better growth of the plants on virgin soils. After these soils had been in use for several years the development of the plants was much poorer. This is undoubtedly due to the continued use of the ammonium sulphate which constitutes the usual form of nitrogen in the fertilizer used in pineapple culture. This results in a highly acid condition of the soil which brings manganese into solution. Certain pineapple soils investigated by us have shown pH values as low as 4.0 and 4.2, and one grower reported the low value of 3.8.

That the fertilizer is responsible for this high acidity is shown by the fact that when samples of soil are taken from the top two inches of the soil near the rows where fertilizer is applied a much lower pH and higher soluble manganese are obtained than when taken from a depth of 6 inches midway between banks. In one instance the first method of sampling showed a pH of 4.2 and water soluble manganese, 134 ppm; while the second method showed a pH of 5.5 and water soluble manganese, 65 ppm. The latter soil sample was much less toxic to the common bean, which was used as a test plant, than the former.

Some preliminary experiments on this soil condition will be described here. In these experiments beans were used as test plants since they show the effects of manganese toxicity in a short time while with pineapple plants the development of symptoms may require several months. At first an attempt was made to antidote the manganese toxicity by adding iron in a soluble form to the soil. A sample of a pineapple soil showing well over 100 ppm water soluble manganese was used for pot cultures. Check cultures without any additions showed severe chlorosis of the bean plants 9 or 10 days from planting. While the seed leaves were not distinctly chlorotic they were a somewhat lighter green than those of normal plants and developed characteristic minute necrotic lesions, dark brown in color, distributed over the entire leaf. Some of these lesions also appeared on the stems and leaf petioles. When, however, the first true or trifoliate leaves appeared they were extremely chlorotic and finally died. The plant, therefore, failed to develop further since the growing point was dead. This is shown in figure 1, culture "A." When humate iron was added to the same soil at the rate of 20 ppm Fe, the same symptoms occurred as in the check plants and it was concluded that iron in this amount was unable to balance the large amount of soluble manganese in the soil.



Next, it was decided to first immobilize the high amount of soluble manganese according to the procedure of Funchess (3). This was done by neutralizing the soil acidity with calcium carbonate and raising the pH to 6.2. To another set of soil cultures both calcium carbonate and humate iron (20 ppm Fe) were added to the soil. The result is shown in figure 1. Without treatment, severe chlorosis followed by necrosis and death occurred as before described. Addition of calcium carbonate to pH 6.2 resulted in the correction of chlorosis and other symptoms of manganese toxicity, and much better growth took place. (Compare in figure 1 the first trifoliolate leaves in culture "B" which are about normal size, with the chlorotic aborted ones in culture "A.") The further addition of soluble iron gave still better growth as will be seen in figure 1, culture "C," where the plants are much taller and the second trifoliolate leaves have formed and developed to considerable size. This latter is important in showing that additional iron has a further effect on the growth even after the chlorotic condition has been corrected. The following measurements were made in connection with the experiment just described.

No.	Treatment	Ave height of plants, 13 days	Ave. width of seed leaves 13 days	Ave length of first trifoliolate leaves 18 days	Ave. height of plants, 18 days	Total dry weight of tops, 43 days
		mm	mm	mm	mm	gms
1	None, check	69.5	28	11	96	0.61
2	CaCO <sub>3</sub> to pH 6.2	70.5	45	47	140	2.05
3	Like 2 but plus 20 ppm Fe as humate	85.0	53	57.5	200	2.60

To show the effect of calcium carbonate on both the pH and soluble manganese content of this acid pineapple soil, an experiment was made. Varying amounts of calcium carbonate were added to a given amount of the soil and thoroughly mixed. The moisture was adjusted to a content about optimum for plant growth. The pH was then determined with the glass electrode, the soluble manganese extracted with distilled water and its amount determined by the potassium periodate method. The results are shown graphically in figure 4. As the amount of calcium carbonate increases and the pH likewise increases, the amount of soluble manganese falls off rapidly until at pH 7.4 it is somewhat less than 20 ppm.

A word should be said at this point as to why raising the pH of a soil which shows no water soluble iron to begin with (and especially with calcium carbonate) should correct a condition which is apparently an iron deficiency chlorosis. It was formerly considered that most cases of this type are associated with high pH and high calcium carbonate. Iron defi-

ciency was not thought of in connection with acid soils. We offer the following explanation. For some reason when these soils become acid, manganese becomes very soluble but at the same time iron remains locked up in an insoluble form or at least does not go into solution to any great extent. The chlorosis in this case is due to manganese toxicity which will be shown later to be equivalent to iron deficiency or lack of balance between the two elements. When the soluble manganese is immobilized by raising the pH with calcium carbonate the chlorosis disappears.

Johnson (19) proposed to explain the action of manganese by its oxidizing effect in the soil. According to his idea, the iron is oxidized to the ferric

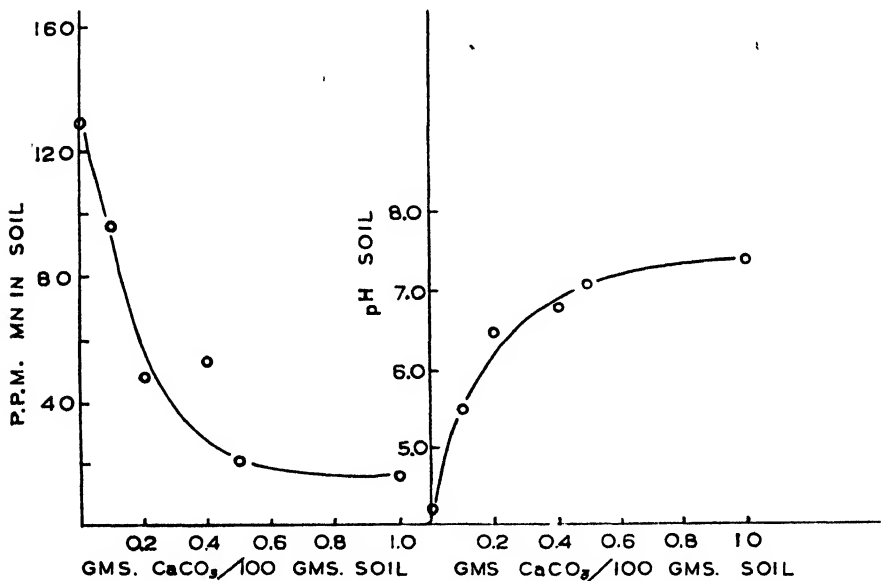


FIG. 4. Effect of calcium carbonate on the soluble manganese and the pH of acid pineapple soil.

condition in which form it is more readily precipitated and hence becomes unavailable to the plant. This hypothesis, however, would hardly apply to these soils of such a high acidity and would not explain why the mere addition of calcium carbonate, as shown above, will largely correct this condition. Furthermore, it would not explain the chlorosis in the solution culture experiments in our work in which the iron always remained in solution as humate iron, regardless of the amount of manganese present.

In regard to iron our tests were made by extracting the soil with distilled water. The negative tests obtained for iron do not mean, of course, that no iron was available to plants. It seems, however, that the available

iron in these soils was relatively low since certain other Puerto Rican soils extracted and tested in the same way have given good positive reactions for iron.

It is, therefore, not always a low concentration of iron that brings about "iron deficiency chlorosis" but the ratio or balance between iron and manganese (or possibly some of the other heavy metals). In this connection, reference should be made to the important observation of Schappelle (29, 30) in his experiments on the effect of minor elements on pineapple plants. He found that if all the usual minor elements were present in the culture solution, normal green plants were produced. If, however, all the minor elements were omitted, green plants with luxuriant vegetative growth were also produced. While these latter plants were abnormal in another way, that they never produced flowers or fruits although "smoked" several times with acetylene gas in solution, they did not develop chlorosis although kept in culture for more than two years. If on the other hand, iron alone were omitted from the solution, marked chlorosis and death of the plants occurred although obviously they had as much iron as those from which all the minor elements were missing.

In one of our own water culture experiments (26-I) it was found that when the nutrient solution was made in tap water, chlorosis and necrosis of bean plants appeared sooner and were more severe, and the dry weights were less when no minor elements were added than in identical solutions made in distilled water. Analysis of these solutions showed that there was slightly more iron in those made with tap water than in those made with distilled water. The amount of manganese was also greater in the case of tap water. In fact, no manganese could be detected where distilled water was used. If we add to the amount of manganese and iron in the solution (that from the water<sup>4</sup> and impurities in the nutrient chemicals) the amount present in the seed, we have:

	ppm	
	Fe	Mn
Cultures made with distilled water .. . . .	0.10	0.15
Cultures made with tap water ... . .	0.12	0.30

This is a case then where iron deficiency chlorosis is more severe with a higher concentration of iron. The apparent anomaly is due to the higher ratio of manganese to iron where tap water was used. Since the relative amounts of manganese and iron in the seed that are in an available form are unknown, this ratio may actually be higher than would appear from

<sup>4</sup> By analysis it was found that the tap water at the Experiment Station at Río Piedras contained 0.02 ppm iron as Fe and 0.1 ppm manganese as Mn.

the figures given above. When 0.5 ppm of manganese was added to cultures identical with the above, severe chlorosis appeared in both cases.

Since this work was completed, an excellent paper by Somers and Shive (33) has appeared in which they have also shown clearly for soybean plants that "iron deficiency chlorosis" is identical with manganese toxicity chlorosis. They also point out that pathological symptoms produced by excessive iron are identical with those produced when manganese is deficient.

The antidoting effect of iron on manganese toxicity had previously been reported in the literature. Among these reports may be mentioned the work of Tottingham and Beck (35) with wheat, Johnson (18) with rice, and Rippel (28) with barley. In all these cases chlorosis caused by manganese in solution cultures was prevented by the addition of iron.

In our investigations iron toxicity was not encountered, either in soil or in water cultures. This, we think, was due primarily to the fact that manganese, due to impurities in nutrient salts and the amounts in seed or seed pieces (pineapple slips), never was at a low enough concentration for iron to become toxic although concentrations as high as 20 and 30 ppm of iron were used.

There is also the possibility that iron toxicity may not have shown up because of: 1—a difference in the reactions of the plants used in this work (bean, tomato and pineapple) compared with the soybean plant used by Somers and Shive; 2—the greater amount of reduction of iron at the high light intensities prevailing in the tropics and; 3—the maintenance of a relatively low pH and the use of inorganic iron by Somers and Shive may have resulted in a higher iron-ion concentration (Hopkins 11) than was realized in the present work at a higher pH with humate iron. A slight indication of iron toxicity was previously noted by Hopkins (12, p. 28 and figure 2). This was in the growth of the common duckweed *Lemna minor*, in various combinations of iron and manganese. Growth with iron but without manganese was slower than where both elements were lacking. The author suggested that it might be due either to a greater proportion of reduced iron or to lack of antagonism.

Further, there is the possibility that in solutions of relatively low pH in which iron is added as a ferrous salt and the solutions frequently changed, the toxicity observed may be ferrous iron toxicity rather than just iron toxicity. The conditions maintained may tend to stabilize iron in the ferrous state.

The purpose in this investigation was, from the beginning, to concentrate mainly on amounts of these elements within their normal range as might be encountered in soil culture, rather than to use highly refined methods and purified chemicals such as are used to bring out deficiency symptoms and to demonstrate the necessity of minor elements. It was proposed to

determine if within these normal limits marked variation in yields could be obtained which would point the way to better crop production. Iron and manganese were chosen because their essential nature for the growth of green plants has been shown beyond doubt (12). In some unpublished work the senior author had also demonstrated for the unicellular alga *Chlorella* sp., that a very important reciprocal relationship between iron and manganese, similar to the above mentioned effect on higher plants, exists. Besides being necessary elements they are two of the most important minor elements from the standpoint of practical agriculture.

#### EXPERIMENTAL METHODS

*Culture solution.* The basic culture solution used in most of these experiments was according to the following formula:

Salt	Formula	
	pH 5.5	Gms./L.
Potassium dihydrogen phosphate . . .	$\text{KH}_2\text{PO}_4$	0.300
Calcium nitrate . . . . .	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	0.500
Magnesium sulphate . . . . .	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.500
Ammonium sulphate . . . . .	$(\text{NH}_4)_2\text{SO}_4$	0.100

Chemicals of CP grade without further purification and distilled water, rain water and tap water were used in making the solutions as indicated in the individual experiments. Iron was added in the form of potassium iron humate prepared according to the method of Horner, Burk and Hoover (13) and manganese as manganous sulphate.

*Culture vessels.* For some of the preliminary experiments and special tests, liter pyrex beakers were fitted with shields to exclude light from the solution and the tops were covered with paraffined cardboard in which holes were made for the plants. The plants were supported with pieces of absorbent cotton. The number of cultures varied in the different experiments and the solutions were changed once a week. For the larger and more exact experiments a series of 42, 20-liter pyrex jars were employed. These were buried in sand in two adjacent benches in the greenhouse to within about 2 inches of the top, the exposed part of which was painted black. The type of cover varied with the kind of plant used. Air from an air-pressure line was constantly bubbled through the solution for the purpose of stirring and aeration. Solutions were changed once a month. The jars were numbered at random so that treatments and replications were also at random. A partial view of this equipment is shown in figure 5.

One experiment (26-I) was carried out in ten subirrigation gravel beds, 6 x 4 x 1 feet deep, each made of sheet metal coated with asphalt paint. In each case the bed was connected with a solution storage tank and a centrifugal motor pump. The pumps were controlled by a time clock in

such a way that the nutrient solution was pumped 6 times a day (twice at night and 4 times in the daytime) to within about 1 inch of the gravel surface. The solution then drained back by gravity into the storage tank. The storage tanks had a capacity of 100 gallons, and 50 gallons of nutrient solution were used for each culture. Detailed description of the apparatus will not be given since it is very similar to that used by Withrow and Biebel (37) and others.

*Observations and measurements.* The cultures were examined frequently for chlorosis, necrosis, general vigor of the plants and any other condition



FIG. 5. Partial view of solution culture equipment used in these investigations. Photograph shows pineapple cultures.

noted. For these items a numerical estimation, usually on the basis of 10 or merely a plus and minus record, was made and since in the large experiments the treatments were at random, the estimation was made without knowing the given treatment. Records were kept of these observations so that, for example, in the case of chlorosis, not only the fact that it occurred but also the time of its appearance and its relative severity were noted. This, combined with the number of plants in a given treatment to show it, gave a rather good measure of the effect of the treatment.

The time of appearance and number of such things as first, second and third trifoliate leaves, tendrils, flower buds, flowers, fruits, etc., were also noted. Measurements of the heights of plants and lengths of the corre-

sponding leaflets for each plant were taken at intervals and finally the plants were harvested and the green and dry weights of tops and roots determined.

#### EXPERIMENTAL RESULTS

##### *Experiment 26-A. Bean*

Bean plants were grown in liter beakers with variable amounts of both manganese and iron merely for the purpose of observing the symptoms of manganese toxicity and the antidoting effect of iron. In the basic culture solution (p. 52) ammonium sulphate was used as the entire source of nitrogen and calcium was furnished by calcium chloride. Although the plants were grown for a period of only 10 days, the final pH of the solutions varied from 3.50 to 3.65.

The following diagram shows the set-up of the experiment and the relative amount of chlorosis after 3 days from planting.

Mn  ppm	Fe			
	3 ppm	6 ppm	9 ppm	27 ppm
2	(4) 0	(5) 0	(6) 0	---
3	(7) 0	(8) 0	(9) 0	---
9	(10) 0	—	(11) 0	(12) 0
20	(1) +++++	(2) +++	(3) ++	---

Culture numbers are given in parenthesis.

This chlorosis which showed up very soon only appeared in solutions having 20 ppm manganese and decreased rapidly as the iron increased from 3 ppm to 9 ppm. At 7 days from planting, chlorosis was still very severe in culture number 1 and remained so until the tenth day when the experiment was terminated. On the other hand, chlorosis in cultures 2 and 3 was less at 7 days so that No. 2 showed only slight chlorosis and No. 3 very slight chlorosis. Both practically recovered from the chlorotic condition by the tenth day. None of the other cultures were chlorotic.

As stated above chlorosis was very severe after 3 days in culture 1. The cotyledons and stems were pale white on two of the seedlings and the seed leaves were chlorotic and stunted. On the other 2 seedlings the stems and cotyledons were partly chlorotic and the seed leaves which were about 1 inch wide showed a chlorotic pattern, white along the midrib and widening out at the tip. The chlorosis was pure white rather than yellow-

ish white. In this culture the roots were normal in appearance and well developed. The extreme chlorosis shown here is thought to be due to the high acidity resulting from the use of ammonium sulphate. In later experiments where calcium nitrate was used such marked symptoms were not found even at 20 ppm manganese and no added iron. The stems and cotyledons were not affected and the seed leaves while often pale green did not show definite chlorosis. However, they often showed minute brown necrotic spots which were always associated with high manganese and low iron.

The roots in all cultures of this experiment appeared to develop normally with good color and abundant fibrous secondary roots. The only abnormality was a slight browning of the root tips in practically all cultures. This was thought to be due to the high acidity in the medium after some growth had taken place. This trouble, however, was less severe in cultures with high manganese and low iron.

Dry weights of the tops and roots were taken at the end of the tenth day. They do not show a definite trend except in the low iron and the high manganese series. At the lowest iron concentration (3 ppm) there is a general decrease in the dry weight of the tops as the manganese increases and at the highest manganese concentrations there is an increase as the iron increases. The results are given below.

Mn	Fe			
	3 ppm	6 ppm	9 ppm	27 ppm
2	(4) 193 47 240	(5) 180.5 41 221.5	(6) 174 42 216	—
3	(7) 170 45.5 215.5	(8) 181 39.5 220.5	(9) 159.5 38.5 198	—
9	(10) 154 44 198	—	(11) 237 62 299	(12) 212.5 61 273.5
20	(1) 165 47.5 212.5	(2) 202 53 255	(3) 256 68 324	—

Dry weights in milligrams, tops above, roots middle, totals below.



The above results show rather definitely the antidoting effect of iron on manganese toxicity and is in line with the effect shown in regard to chlorosis.

Several things are evident from this experiment. 1—Manganese at 20 ppm is injurious to the tops of bean plants at 3 ppm iron. This is shown both by severe chlorosis and decreased yield. 2—As the iron content is increased the injury becomes less and the dry weight increases. 3—Even with severe injury to the tops the roots appear to be normal. 4—Manganese injury to the tops is caused by an upset in the chlorophyll mechanism. 5—Iron in the form of potassium iron humate in concentrations up to 27 ppm is not injurious to bean plants with manganese at 9 ppm.

*Experiment 26-B. Bean*

This was also a preliminary experiment for the purpose of observing chlorosis. Twelve cultures all containing 20 ppm manganese were set up. The iron was varied from none to 30 ppm Fe. Ammonium sulphate was again used as the main source of nitrogen but the solutions were kept from becoming acid by subsequent additions of sodium nitrate. In this experiment there was no chlorosis of the cotyledons and stems but it showed up to a slight extent on some of the seed leaves. However, chlorosis was very severe on the first trifoliate leaves at low iron concentrations. Observations on chlorosis, 12 days after planting were as follows.

Culture Number	Iron	Chlorosis on seed leaves	Chlorosis on trifoliate leaves
	<i>ppm</i>		
1	0	slight	++
2	2	slight	+
3	4	slight	++
4	6	slight	++
5	8	slight on one plant	++
6	10	none	++
7	12	none	++++
8	14	none	+++
9	16	none	+
10	20	none	none
11	24	none	none
12	30	none	none

Culture No. 7 shows the most striking manganese chlorosis on the trifoliate leaves. However, these leaves were about  $1\frac{1}{4}$  inches long, while in cultures 1 to 6 they were badly stunted and hence more affected by man-

ganese toxicity. It is clearly evident from this that manganese toxicity is counteracted by iron and that under the conditions of this experiment 20 ppm of manganese require about 16 ppm of iron to antidote its effect as far as chlorosis is concerned. After 15 days necrosis was observed to follow chlorosis in cultures with low amounts of iron. At this time culture 12 also showed slight but definite chlorosis. Whether this was a case of iron toxicity or was due to excessive amounts of both elements is uncertain. Cultures 10 and 11 did not show any evidence of chlorosis.

#### *Experiment 26-C. Bean*

This was a preliminary experiment designed to find out if the humate fraction of the humate iron had any effect on manganese toxicity apart from the iron. Cultures were set up in the same manner as in the previous experiment, but instead of allowing the humate fraction to vary with the iron, as in previous tests, it was kept at a constant amount equivalent to that in the culture with the highest amount of iron (30 ppm). As before, all cultures had 20 ppm manganese.

Ten days after planting there was some evidence of chlorosis on the first trifoliate leaves in all cultures, which varied from very severe in culture 1 in which the leaves were reduced in size, to very slight in 10, 11 and 12. There was a uniform decrease in severity of chlorosis as the iron content increased. The plants in culture 1 were smaller, the seed leaves were smaller and definitely paler in color and the first trifoliate leaves were smaller, slower to open and, as before stated, very chlorotic. There was a marked contrast between culture 1 and culture 2 since in the latter the trifoliate leaves were open and measured from one to several centimeters across, while those of culture 1 were not yet fully open.

It was further found that with higher amounts of iron chlorosis which appeared in the early stages of growth would disappear often within as short a period as two days. This effect was observed later with tomato plants. Without frequent observations the symptoms could easily be missed in certain cultures. This was interpreted as indicating a relatively more rapid absorption of manganese than iron in the early stages. Later, when more iron was taken up, the toxic effect of manganese was antidoted. This experiment again showed the antidoting effect of iron on manganese, and further, that the humate fraction of the humate iron had no effect in correcting manganese toxicity.

An interesting phenomenon which will be discussed more fully later in this paper, was observed in this experiment at "zero" iron (culture 1). On bright sunny days the seed leaves of plants in this culture were oriented in such a way that the leaf surfaces were parallel to the sun's rays, while on

cloudy or rainy days these same leaves resumed their normal horizontal positions. This is apparently an adaptive mechanism which prevents severe injury at high light intensity. Culture 2, which differed from 1 in that it received 2 ppm iron, did not show this nor any of the other cultures in the experiment. The result was confirmed in a later experiment (26-F).

In explanation of the fact that no definite chlorosis of seed leaves appears in most of these experiments when the trifoliate leaves may show severe injury, it is suggested that the seed leaves may have a considerable portion of the reserve iron of the seed or that the reserve iron of the cotyledons is translocated mainly into the seed leaves and there antidotes to a large extent the manganese. With greater toxicity, of course, marked chlorosis appears on the stems, cotyledons and seed leaves which may be pure white.

#### *Experiment 26-F. Bean*

The purpose of this experiment, set up about 6 months later than No. 26-C, was to check on the matter of leaf orientation in the light, to check again the effect of the humate fraction apart from the iron and also to obtain more data on the antagonistic action of iron towards manganese. The set-up was as follows.

Culture Number	Iron	Manganese	Sol 3-A	Sol 3-B
	<i>ppm</i>	<i>ppm</i>	<i>ml per liter</i>	<i>ml per liter</i>
1	0	20	0	12
2	2	20	2	10
3	4	20	4	8
4	8	20	8	4
5	12	20	12	0

Solution of 3-A was a potassium iron humate solution while 3-B was the same humate preparation without iron. Hence, there was the same amount of humate in each culture.

On the fourth day after planting, although the light in the greenhouse was not very intense, quite a number of the seed leaves in each of the cultures 1 to 4 were elevated from the horizontal about 20 to 30 degrees. All seed leaves in culture 5 were horizontal. On the fifth day, which was partly cloudy, seed leaves in all cultures were horizontal. The same was true on the eighth day. On the tenth day two seed leaves in culture 1 were found to be vertical in a light of medium intensity while all other seed leaves were horizontal.

On the twelfth day a total of six seed leaves in culture 1 were vertical in the sunlight. The other two which did not respond to the light were somewhat wilted. Movement of these leaves was observed, during the

time of taking notes, from an angle of about 60 degrees from the horizontal to the vertical. This was caused by the sun coming from behind a cloud. Again, the seed leaves in all other cultures were horizontal. Later on a sunny morning all of the eight seed leaves of culture 1 were vertical and this time, as a cloud passed over the sun, they were seen to move from the vertical to an angle of about 60 degrees in one-half hour. With continued



FIG. 6. Phototropism of seed leaves of bean plants caused by manganese toxicity. Above and left—20 ppm manganese and no iron; leaves are parallel to sun's rays. Right—20 ppm manganese plus 2 ppm iron; leaves are horizontal. Both cultures in sunlight. Below—The same 2 cultures after shading; the leaves are horizontal in both cultures.

cloudiness for another hour they all became perfectly horizontal. These movements were frequently observed and also produced at will by artificial shading. The effect is illustrated in figure 6. At the top are shown cultures 1 and 2 on a sunny day; the seed leaves of culture 1 are vertical while those of 2, which differs only in that it contains 2 ppm iron, are horizontal. The same cultures are shown below after they were artificially shaded for about 1 hour. The seed leaves are horizontal in both cultures.

As mentioned before, this phototropism is apparently a mechanism which prevents excessive injury to the leaves at high light intensity. In explanation of the fact that on the fourth day phototropic response was exhibited by seed leaves in all cultures except No. 5, it should be said that in cultures 2 to 4 and to some extent in 5 there was chlorosis in the early stages with subsequent recovery.

In regard to chlorosis in this experiment it was again most severe where no iron was added, and gradually decreased as the iron content of the solution increased. The same was true of necrosis and other symptoms of manganese toxicity. At 4 days the cotyledons of one plant in culture 1 showed chlorosis and some of the seed leaves in both cultures 1 and 2 had chlorotic patterns. Seed leaves in culture 5 at this time were a good green. The first trifoliolate leaves in culture 1 were very small, did not fully open and finally became necrotic and died, while in the other cultures, although they showed chlorosis which varied inversely with the amount of iron, this disappeared about 14 days from planting. The effect of treatment on development is very well shown in figure 6 where it will be seen that both the first and second trifoliolate leaves have formed and opened in the plants of culture 2 while in culture 1 only seed leaves are in evidence. The following counts and measurements were made of the plants in this experiment.

Culture number	Iron	Ave. width seed leaves 10 days	Ave. length 1st trifoliolate leaves 17 days	No. 3rd trifoliolate leaves opened 17 days	Oven dry wts. per plant, gms. 21 days		
					Top	Root	Total
	<i>ppm</i>	<i>mm</i>	<i>mm</i>				
1	0	63	20*	0	0.317	0.090	0.407
2	2	72	90	1	0.740	0.265	1.005
3	4	77	91	2	0.959	0.284	1.243
4	8	71	97	3	0.915	0.245	1.160
5	12	70	100	4	1.110	0.262	1.372

\* Leaves dead.

In the above experiment the antagonistic action of iron towards manganese is shown by variation in: (1) chlorosis of seed leaves; (2) chlorosis and necrosis of the first trifoliolate leaves; (3) rate of development and size of the trifoliolate leaves; (4) size of the seed leaves and dry weights of tops and roots of the plants. It is also shown in a general way by their phototropic response. In regard to culture 3, which is out of line in some respects, it should be noted that only two of the 4 plants developed in this culture and hence there was less competition.

*Experiment 26-G. Bean*

This time calcium nitrate was used as the main source of nitrogen although some ammonium sulphate was used for the purpose of physiological buffering. Iron and manganese concentrations were varied simultaneously. The cultures were placed at random on the greenhouse bench. Five days after planting, the seed leaves which opened out were carefully measured but showed very little difference in size. At nine days, when the first trifoliate leaves were opened in all cultures but the check, they showed marked correlation of chlorosis with the treatment. In the table below, the plan of the experiment is shown and the relative amount of chlorosis at nine days is indicated by plus and minus signs.

Mn	Fe			
	2	5	10	20
<i>ppm</i>				
2	—	—	—	—
5	++	+	—	—
10	+++	++	+	+
20	++++	+++	—	++

“Check” with the same macro elements but *no Fe or Mn*. First trifoliate leaves not yet open.

The above shows clearly the interrelationship of iron and manganese in bean plants. With a high ratio of iron to manganese there is very little chlorosis and the reverse is true with high manganese and low iron. If a diagonal is drawn from the upper left to the lower right of the diagram, only one plus sign appears above the diagonal while 19 appear below. Plants in the check culture without either iron or manganese added showed the severest symptoms of toxicity. The first trifoliate leaves of these plants did not open until much later than those in the other cultures and they were very small and completely chlorotic. This was later found to be due to manganese in the tap water used in these cultures (see Experiment 26-I).

Measurements of the length of the central leaflet of each trifoliate leaf were made on the ninth and on the eleventh day. The averages for each of two categories and the check are as follows:

	9 day	11 day
	<i>mm</i>	<i>mm</i>
High iron-low manganese (upper right).....	51.2	85.0
High manganese-low iron (lower left).....	44.1	72.7
Check plants.....	0.0	23.0

Dry weight determinations of the tops and roots made after 31 days growth are given below.

Mn  ppm	Fe				
	2 ppm	5 ppm	10 ppm	20 ppm	"Check"
2	4.77	4.09	4.58	4.86	2.01
	2.32	1.97	1.76	1.56	1.34
	7.09	6.06	6.34	6.42	3.35
5	3.60	3.65	4.70	4.58	
	1.64	1.67	1.69	1.87	
	4.94	5.32	6.39	6.46	
10	3.96	4.68	4.36	4.89	
	1.54	1.84	2.20	1.58	
	5.50	6.52	6.56	6.47	
20	3.36	4.16	4.25	4.75	
	1.42	1.70	1.50	2.00	
	4.78	5.86	5.75	6.71	

Note. Weights are in grams for 4 plants in each culture. In each square the dry weight of tops is at the top, roots in the middle and the total below.

Although the differences in the dry weights are not large, there are definite trends. At 5, 10 and 20 ppm manganese the dry weight increases as the iron increases, and at 2 ppm iron they decrease as the manganese increases. These yields, in connection with the previous observations on chlorosis and the development of the trifoliate leaves, show very well the antagonistic effect of iron on manganese and bring out the fact, as did the other experiments, that the two elements cannot be considered apart from each other in judging their physiological action. For example, a very slight amount of manganese may be much more toxic than 10 or 20 ppm when not balanced by sufficient iron. This is brought out by the check plants where the concentrations of manganese and iron were 0.30 and 0.12 ppm respectively.

An observation made in the course of this experiment may have some practical importance. Leaves showing manganese chlorosis were placed between blotters to dry. Several days later it was noted that the original light colored areas had turned very dark, almost black. This was apparently due to rapid oxidase action in the presence of relatively large amounts of manganese. This is similar to the observation reported by Kelley (20) for pineapple leaf tissue, and may also be related to "black tobacco" reported from Connecticut by Le Compte (23) as occurring in tobacco

plants with higher than the average manganese and iron contents. The leaves from this tobacco cure very dark brown with a blue-gray or purple-gray hue.

### *Experiment 26-H. Bean*

The purpose of this experiment was to study the antagonistic action of iron on manganese, this time on a larger scale. A greater number of treatments, larger culture vessels and a larger number of plants per culture were used. The 20-liter pyrex culture jars described on page 52 were used. Nineteen liters of culture solution were placed in each jar and the solutions were constantly aerated. Seven bean seedlings were originally planted, and after several days when a few abnormal ones were eliminated, the number was reduced to six normal seedlings in each culture. The solution, as regards the macro elements, was according to the formula given on page 52. The solutions were made with tap water. There were seven different concentrations of iron, and for each concentration of iron six concentrations of manganese. Thus a total of 42 cultures resulted which were randomized as before described. The following diagram gives the details of the set-up.

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
Culture Numbers							
ppm							
0	1	2	3	4	5	6	7
1	8	9	10	11	12	13	14
2	15	16	17	18	19	20	21
5	22	23	24	25	26	27	28
10	29	30	31	32	33	34	35
15	36	37	38	39	40	41	42

Various observations, measurements and estimations of general development were made during the course of the experiments and finally determinations of fresh and dry weights of the plants were carried out. In tables 1 to 10, arranged similarly to the above, these are given. As will be seen, there is a consistent advantage from the standpoint of the plant, for each item, as the iron content increases from 0 to 15 ppm, and a corresponding disadvantage as the manganese is increased. The relative effect of each of the two factors (iron and manganese) is dependent on the concentration of the other.

In Experiment 26-H, as shown by the data in tables 1 to 11, there was excellent growth in those cultures with a high iron concentration in relation to the manganese, i.e., in cultures represented in the upper and right area of the above diagrams (tables). From the standpoint of the appearance



of the plants, the best culture was number five, with no added manganese and 5 ppm iron. At 13 days it was rated as by far the best, with deep green

TABLE 1  
*Effect of iron and manganese on chlorosis after eight days. Experiment 26-H*

Mn ppm	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
0	+++++	+++					
1	+++++	+++++	+++++	+++++	+++	+	
2	+++++	+++	+++	+++++	+++	++	++
5	+++++	+++++	+++	+++++	+++++	+++++	++
10	+++++	+++++	+++++	+++++	+++++	+++	+++++
15	+++++	+++++	+++++	+++++	++	+++	++++

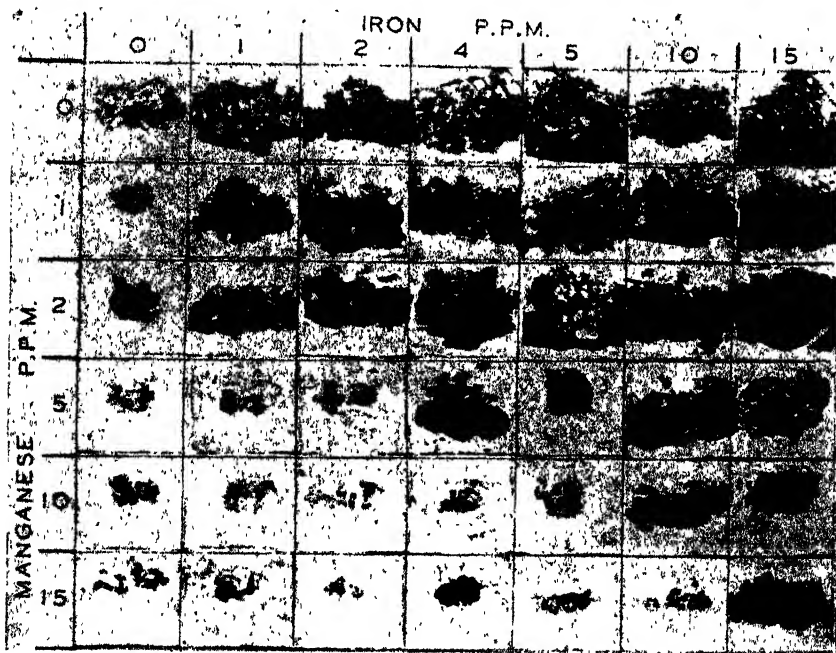


FIG. 7. Effect of the simultaneous variation of manganese and iron on the growth of bean plants. Experiment 26-H, 20 days after planting. Photographs of cultures are arranged according to treatment and extraneous background has been blocked out. The purpose is to show the general effect of treatment on growth rather than detail.

foliage and the third set of trifoliate leaves beginning to open. At 16 days the growth of the six plants in this culture was so luxuriant as to

more than cover the top of the culture jar. It was also the first culture in which flowers opened, but was only fourth in respect to the total amount of dry matter produced (table 11). A discussion of the various items in regard to the plants follows.

*Chlorosis.* From table 1 it is seen that at 8 days a large number of the cultures showed more or less chlorosis. Only 5 cultures at zero manganese and higher amounts of iron showed none. However, shortly thereafter many of the treatments with high iron and low manganese recovered from this initial chlorosis so that at 16 days very few plants in the high-iron low-

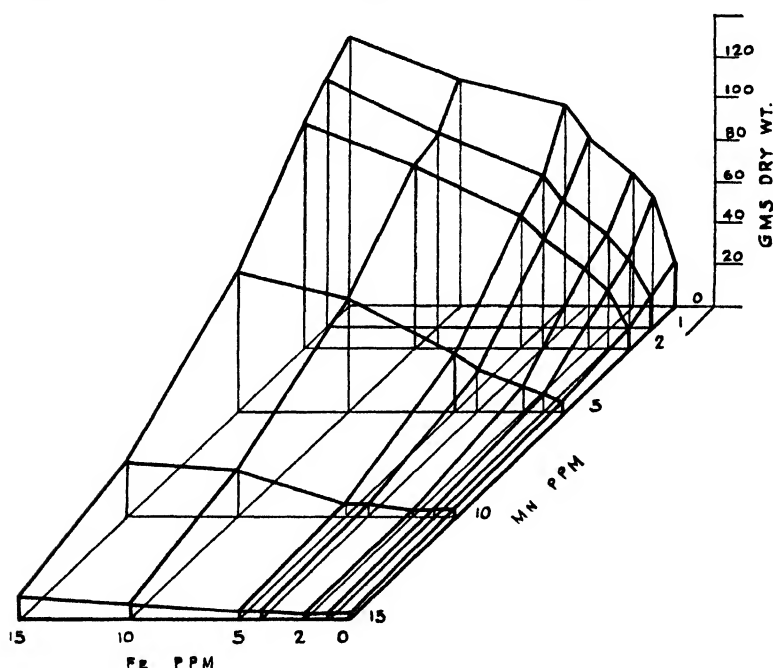


FIG. 8. Effect of simultaneous variation of manganese and iron on the total dry weights of bean plants, represented as a three-dimensional figure. Experiment 26-H.

manganese group (above a diagonal drawn from the upper left to the lower right of the diagram, figure 8) showed no chlorosis, while practically all those below the diagonal were still chlorotic. This was taken to mean, in the first case, that in the early stages of growth manganese was absorbed faster than iron, and later, when more iron was absorbed, the toxic effect of manganese was antidoted and chlorosis disappeared. On the other hand, in the second case, enough iron was not absorbed to antidote the manganese because of an insufficiency of the former and an abundance of the latter in the substrate. In the first case the injury was slight and the condition still reversible, but in the second case it was severe, irreversible,

and followed by necrosis of the tissue and final death of the plant. The general trends are: (1) the higher the iron the less chlorosis; (2) the higher the manganese the more chlorosis; and (3) it requires more manganese to bring about chlorosis as the iron concentration increases. The effect shown by culture 1 without either iron or manganese added has already been explained; the solution contained manganese impurities in small amounts from the tap water and from the ordinary CP chemicals in such amounts that the iron impurities were insufficient for proper balance. Therefore, these plants were severely affected. When iron alone is added at 1 ppm (culture 2) almost normal growth with only slight chlorosis is obtained. No evidence of iron toxicity as described by Somers and Shive (33) was indicated at the highest iron and lowest manganese concentrations used (culture 7) by any type of chlorosis. It is possible that enough

TABLE 2

*Effect of iron and manganese on the length of central leaflet of first trifoliolate leaf.  
Lengths in millimeters. Eight days*

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0	29	34	36.5	37	35	29	37
1	17	22	26	26	25	30	35
2	11	21	29	25	30	26	32
5	16	20	22	18.5	22.5	25	24
10	17	18.5	13	17	17.8	18	18
15	10	10	14.5	10	21	10	18

manganese impurities were present in the "zero" manganese cultures to balance the highest iron concentration used.

*Necrosis.* This is another measure of the relative effect of the various combinations of iron and manganese in this experiment, and is, of course, closely related to chlorosis as above mentioned. One type of necrosis is that shown on the seed leaves of bean plants, and consists of minute brown spots usually not more than a millimeter in diameter scattered over the leaf and often on the leaf petioles. Time did not permit a careful study of this, but it is apparently a definite symptom of manganese toxicity which occurs when the plants are quite severely affected. This is brought out in table 3 where it was recorded only in cultures with the highest manganese and the lowest iron concentrations. Why manganese injury takes this particular form and only on the seed leaves, is difficult to answer without further study. Only in a few cases, such as were found in some of the preliminary experiments, were chlorotic patterns followed by irregular

necrotic areas seen on seed leaves. It is suggested that it may be related to a particular kind of distribution of the iron which is translocated from the cotyledons into these leaves.

TABLE 3

*Effect of iron and manganese on the number of cultures showing the formation of minute brown necrotic lesions on seed leaves. Nine days*

Mn	Fe					
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	15 ppm
ppm						
0						
1						
2	+					
5	+	+				
10	+	+	+	+		
15	+	+	+	+		+

TABLE 4

*Effect of iron and manganese on the number of first trifoliate leaves showing some necrosis. Nine days*

Mn	Fe					
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	15 ppm
ppm						
0	+					
1	++++	+	++++	+++		
2	++	++++	++	++		
5	+				+	++
	++++	+++	+++	+	++++	++++
10	+			++		
	++++	+++	++++	++++	+++	+
15	++	+			++	
	++++	++++	+++	++++	++++	+++

The number of first trifoliate leaves in each culture which showed any necrosis at 9 days is given in table 4. Here again, as for chlorosis, the greatest proportion was in the cultures with high manganese and low iron. The necrosis was characterized by irregular shaped brown areas on the trifoliate leaves. In some cases the entire leaf was killed preventing any

further development of the plant, while in others it was slight and the plant continued to grow. The general trends are the same as those just discussed for chlorosis.

*Leaf size and general development of plants.* A numerical expression of the general development of the plants was obtained by measuring the length of the central leaflet of each first trifoliate leaf from the base of the pedicel to the tip. The figures which are shown in table 2 are each the average length of the 6 leaflets for the corresponding culture. An average length of about 10 mm indicates that the leaf had formed but had not yet opened. While there is some variation it is obvious that the lengths of

TABLE 5

*Effect of iron and manganese on susceptibility of the seed leaves to sunscald. Eleven days*

Mn	Fe					
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm
ppm						
0						+
1	+					
2						
5						
10	++++	+++	++ ++++	+	+++	
15		+ ++++ ++++	++ ++++ ++++			++++
	++					

these leaflets vary directly with the iron concentration and inversely with the manganese concentration.<sup>5</sup>

At 12 days a count was made of the number of second trifoliate open leaves (table 6). The largest number was opened in cultures with high iron and low manganese. Likewise, at 22 days the number of cultures in which tendrils had formed were recorded (table 9). These were all in the upper right half of the diagram. Table 10 gives a record of the number of flowers per culture, and here practically all are in the upper right part of

<sup>5</sup> Analysis of variance of the data from table 2 gave the following results:

	Degrees of freedom	"F" value Snedecor (32)
Variance between iron.....	6	5.02 highly significant
Variance between manganese.....	5	28.55 highly significant

TABLE 6

*Effect of iron and manganese on the number of second trifoliate open leaves at 12 days*

Mn ppm	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
0		++ ++++	++ ++++	++ ++++	++ ++++	++ ++++	++ ++++
1	++	+++	+ ++++	+++	+ ++++	++ ++++	++ ++++
2	++	++++	+ ++++	++++	++ ++++	++ ++++	++ ++++
5			++ ++++	++ ++++	+++	++ ++++	++ ++++
10						+++	+++
15							++++

TABLE 7

*Effect of iron and manganese on the general development and vigor of the plants at 16 days. Evaluated on the basis of 10 = the best*

Mn ppm	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
0		++++ ++++	++++ ++++	++ ++++	++ ++++ ++++	+ ++++ ++++	+ ++++ ++++
1		++	+ ++++	++++	++++	++++ ++++	++++ ++++
2	+	+++	++	++++	++++	+++ ++++	++ ++++
5			++	++		++++	++++
10						+	+
15			+			+	++

the table. It is not intended to imply a specific effect of the treatments on reproduction, but merely to assume the effect to be the result of treatments on the general development of the plants. Table 7 gives an estimate of

the general development and vigor of the plants at 16 days from planting. At 20 days from planting each culture was photographed with the camera always placed at the same distance from the culture. The photographs obtained were arranged according to the diagram of this experiment, and after blocking out extraneous background with white ink, the whole was rephotographed to a smaller size. The result is presented in figure 7 which shows very strikingly the general effect of the treatments on the growth of bean plants. In the lower left portion of the figure the small and extremely chlorotic plants scarcely show up against the white background.

*Sunscald.* Due to somewhat excessive temperature over a week-end when the greenhouse was closed up, there was some injury from sunscald. This was not severe and occurred only on the seed leaves. As will be seen in table 5, this was present mostly at the two highest manganese concentrations and decreased as the iron content of the substrate increased. While an incidental observation, it is important in showing that iron exerts a protective action against high temperature just as it does against intense light. A further observation in regard to sunscald will be given in connection with Experiment 26-I.

*Dry weights of plants.* The plants were harvested after 44 days from planting. At this time many bean pods had developed to a good size in the high-iron low-manganese cultures. The dry weights of the tops, roots and their totals are given in table 11. In all cases there is an increase with increasing iron and a decrease with increasing manganese, and the cultures represented in the upper right part of the diagram are seen to have produced by far a greater total dry weight than those in the lower left. The ratio is more than 6 to 1. For the tops alone it is almost 7 to 1 and for the roots about 5 to 1. Because of the fact that the treatments were not replicated and, therefore, considerable variation might be assumed, we feel that too much weight should not be assigned to minor deviations from the general trends.

The data for the total dry weights are shown graphically in the form of a solid figure (figure 8) where the iron and manganese concentrations are given along two sides of the base and the dry weights represented by vertical distances from the base. The values used to construct this figure were obtained from smoothed curves of the actual data. From this graph the simultaneous effect of iron and manganese on growth can be clearly seen. If the total dry weights at each iron concentration (disregarding variation in manganese) are averaged, and the same is done for manganese, the two graphs shown in figure 9 can be constructed. This gives an overall picture of the effect of the two elements.

A preliminary statistical analysis of the data for total dry weights was made using Student's method and pairing first the values for the various iron concentrations in each case with "zero" iron at all the different man-

ganese concentrations. The odds of significance that the growth was greater, increased as the iron concentration increased. High significance was found at 4, 5, 10, and 15 ppm iron. The same was true for manganese: significant decreases in growth were found at 2, 5, 10 and 15 ppm manganese over "zero" manganese. The odds increased as the concentration of manganese increased. Later the data as a whole were tested by Fishers' method of analysis of variance and found to be highly significant.<sup>6</sup>

The ratio of dry weight of tops to the dry weight of roots was calculated for each culture, and while the general trend is the same as for the absolute

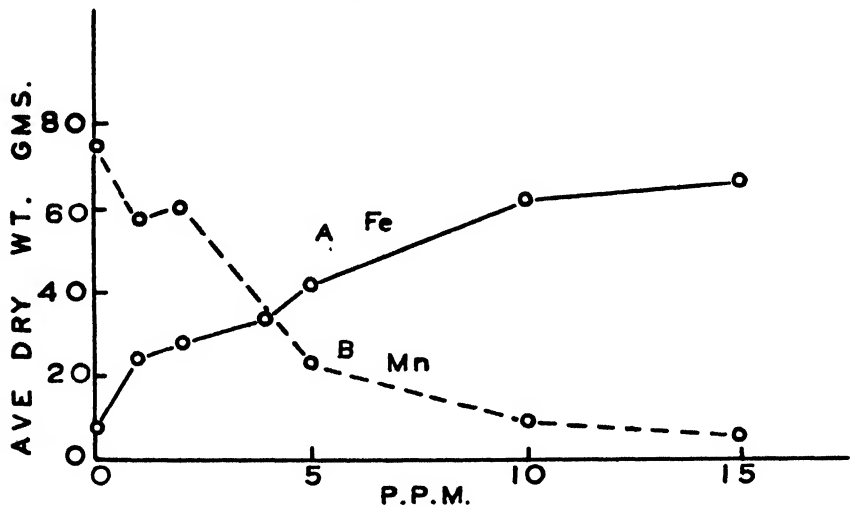


FIG. 9. Effect of manganese and iron on the growth of bean plants. Experiment 26-H. A—Average total dry weights at various iron concentrations disregarding variation in manganese. B—The same for various manganese concentrations disregarding variation in iron.

weights, the variation is such as to cast some doubt on the significance of this. It suggests, however, that iron and manganese have a relatively greater effect on the tops than on the roots.

<sup>6</sup> The calculation was made by Mr. K. W. Loucks of the Florida Citrus Experiment Station with the following results:

Dry weights of Iron	Iron		Manganese	
	Degrees of Freedom	"F" value	Degrees of Freedom	"F" value
Tops and roots.....	6	6.39	5	12.82
Tops.....	6	6.02	5	12.50
Roots.....	6	5.01	5	8.60

All "F" values are highly significant. (Snedecor, 32.)



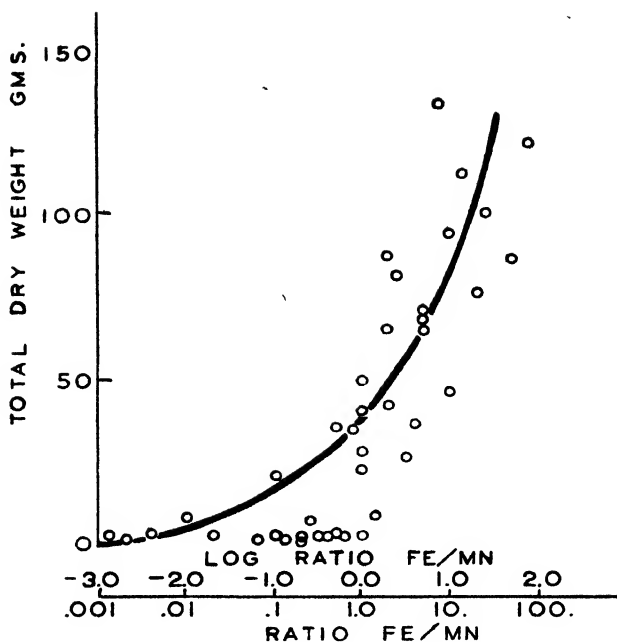


FIG. 10. Effect of the Fe/Mn ratio on the growth of bean plants. Experiment 26-H

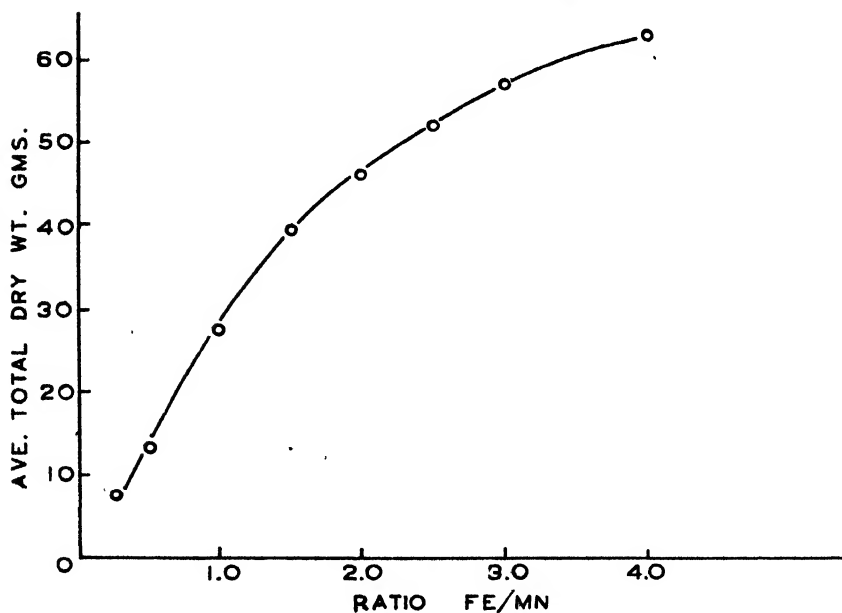


FIG. 11. Average total dry weight for each Fe/Mn ratio plotted against the ratio. Experiment 26-H

*The ratio iron-manganese.* If the actual values for total dry weight are plotted against the ratio Fe/Mn expressed in logarithmic form so as to include the wide range of ratios in one graph, there is much variation of the points, from a smooth curve, as shown in figure 10. There is no doubt, however, of a general increase in total dry weight with an increase in the Fe/Mn ratio from 0.001 to 100. However, the variation mentioned suggests that another factor, namely, the total concentration of the two ele-

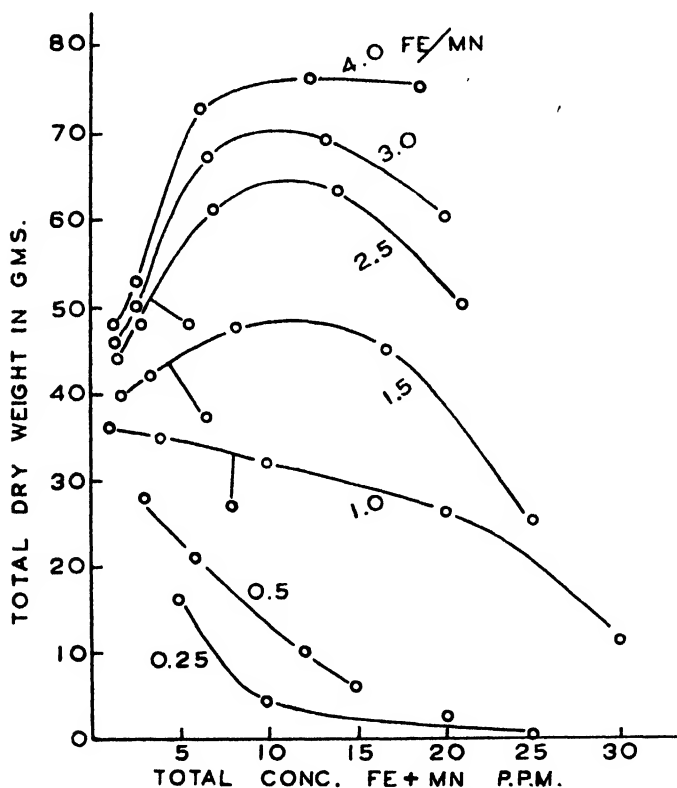


FIG. 12. Effect on bean plants of total concentration of iron plus manganese at various Fe/Mn ratios. Experiment 26-H.

ments, may also be involved. This idea was tested further by arranging values from smoothed curves in various groups, each with a different Fe/Mn ratio, from 0.25 to 4.0, but within each group a variable total amount of iron plus manganese. The average values for these groups plot out as a smooth curve showing a marked increase in dry weight with increase in the ratio (figure 11). Within any given group, however, that is, at a given ratio, the dry weight varies in a rather consistent manner with the combined amount of iron and manganese (figure 12). At the ratios Fe/Mn

0.25 and 0.5 there is a rapid decrease as the total concentrations of these elements increase. At a ratio of 1.0 the decrease is more gradual, while at the higher ratios the dry weight passes through a maximum and then declines, except at 4.0, where there is an increase and no decrease. Therefore, we conclude that under the conditions of this experiment the ratio of iron to manganese is not the only factor involved. The total amount of iron plus manganese is also important. Both are factors in growth, and under certain conditions, even when the ratio is kept the same, increasing amounts of manganese exert a greater toxicity.

The results of an experiment of this type are best depicted as a solid figure such as is shown for the total dry weights (figure 8). However, a brief summary may be obtained by averaging or totalling the results for high-iron low-manganese (upper right of diagram) and comparing with low-iron high-manganese as follows:

Item	High-iron low-manganese	Low-iron high-manganese
Chlorosis, 8 days .....	32	59
Chlorosis, 16 days .....	57	183
Chlorosis, 22 days .....	8	21
Chlorosis, 27 days .....	4	18
Chlorosis, 34 days .....	5	17
Necrosis, seed leaves, 9 days .....	1	11
Necrosis, first trifoliolate leaves, 9 days .....	30	70
Length, first trifoliolate leaves, 8 days .....	28	17
Sunscald, seed leaves, 11 days .....	1	44
General development, 16 days .....	115	13
Number second trifoliolate leaves, 12 days .....	107	28
Number tendrils, 22 days .....	11	0
Number flowers, 36 days .....	279	5
Dry weight, tops, 44 days .....	1124	166
Dry weight, roots, 44 days .....	274	61
Total dry weights, 44 days .....	1397	228

*Conclusions.* For all of the above expressions of development and growth, striking correlation with treatment is shown. The toxic action of manganese and its antagonism by iron are clearly brought out. Roots are relatively less affected by treatment than are the tops. This points to the possibility that the chlorophyll mechanism or photosynthesis, or both, are involved, directly or indirectly. Under the conditions of this experiment mutual antagonism is not evident, only the antidoting effect of iron on manganese. There is a general correlation of the Fe/Mn ratio with growth, but the results also indicate that the total concentration of these elements is also important. The experiment suggests that both a

high ratio of iron to manganese and a relatively high concentration of iron are desirable for good growth of green plants.

*Experiment 26-I. Bean*

This experiment was set up in two parts: A, to test again the effect of the simultaneous variation of iron and manganese, and B, to compare the effect of the use of tap water in the preparation of the culture solution with

TABLE 8

*Effect of iron and manganese on chlorosis at 18 days. Evaluated on the basis of 10 = the most chlorotic*

Mn ppm	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
0	++ ++++ ++++	+		+			
1	++ ++++ ++++	++++ ++++	++	++++	++++		
2	++ ++++ ++++	++ ++++	+ ++++	++	++	+	+
5	++ ++++ ++++	++ ++++ ++++	+ ++++	+++	++ ++++ ++++	++	+++
10	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	+ ++++ ++++	+++ ++++	+ ++++ ++++
15	+ ++++ ++++	+ ++++ ++++	+ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++ ++++ ++++	++++ ++++ ++++

that of distilled water. Liter beakers were used as culture vessels. The treatments are shown in table 12 which also gives the result of observations on chlorosis and necrosis 18 days after planting. The oven dry weights are shown in table 13.

Part "A" of this experiment shows the same effect as previous tests as regards chlorosis and necrosis. It is seen from the results obtained in Part "B" that the culture solution made with tap water gave a markedly

lower yield than that made with distilled water when neither iron nor manganese was added. This is caused by the fact, previously discussed, that tap water contained 0.1 ppm manganese and only 0.02 ppm iron as impurities (see discussion on pages 50 and 51). The addition of 0.5 ppm manganese accentuated the condition in the case of tap water causing necrosis as well as chlorosis and reducing markedly the dry weight. In the case of distilled water a toxic condition is brought about by this amount

TABLE 9

*Effect of iron and manganese on the number of cultures in which tendrils had formed at 22 days*

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		+	+	+	+	+	+
1			+		+	+	+
2							+
5							
10							
15							

TABLE 10

*Effect of iron and manganese on the number of flowers per culture at 36 days*

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
ppm							
0		13	6		65*	38	32
1		1	11		2	17	22
2			2		4	14	34
5				2		3	7
10						2	
15							

\* In this culture (No. 5) many bean pods had formed at this time.

of manganese. When iron is added at the rate of 5 ppm normal growth results in both cases either without manganese or with 0.5 ppm.

#### *Experiment 26-K. Tomato*

This experiment is recorded in this order, since it constitutes a repetition of part "B" of the previous one (26-I), only using tomatoes in place of beans. The purpose was to test the sensitivity of tomato plants to man-

ganese impurities in the tap water just as was done with beans. At the time it was set up, observations on Experiment 26-J had already shown that tomato seedlings were more sensitive (or reacted sooner) to manganese toxicity than beans. This was probably due to a smaller iron reserve in the seed. The variety "Marglobe" was used and one seedling was placed in each culture. Liter beakers were employed as culture vessels. Observa-

TABLE 11

*Effect of iron and manganese on yield. Oven-dry weights per culture (6 plants) at 44 days, in grams*

Mn	Fe						
	0 ppm	1 ppm	2 ppm	4 ppm	5 ppm	10 ppm	15 ppm
Tops							
ppm							
0	13.0	59.2	38.1	66.0	80.7	76.7	95.7
1	2.6	22.6	55.5	27.6	54.3	77.0	90.5
2	6.7	30.0	36.5	30.0	65.5	55.5	106.2
5	2.6	2.2	2.6	24.0	2.2	77.0	19.0
10	1.0	2.5	1.1	2.1	3.0	22.0	7.0
15	2.6	1.9	1.3	5.0	1.8	2.1	18.0
Roots							
0	8.4	11.8	9.6	11.7	19.3	9.5	25.8
1	0.8	5.5	10.6	9.0	10.4	17.5	21.5
2	3.1	6.3	13.5	13.6	15.7	12.5	28.2
5	1.1	0.6	0.8	11.3	0.9	11.8	8.5
10	0.9	1.3	0.4	0.8	1.2	18.5	2.2
15	0.8	0.4	0.4	2.4	0.4	0.4	5.2
Totals							
0	21.4	71.0	47.7	77.7	100.0	86.2	121.5
1	3.4	28.2	66.1	36.6	64.7	94.5	112.0
2	9.8	36.3	50.0	43.6	81.2	68.0	134.4
5	3.8	2.7	3.4	35.3	3.1	88.8	27.5
10	1.9	3.8	1.5	2.9	4.2	40.5	9.2
15	3.4	1.4	1.7	7.4	3.2	2.5	23.2

tions on chlorosis are given for 6, 10 and 17 days in table 14. Just as in the case of bean plants, when tap water was used to prepare the culture solution and no iron or manganese was added the tomato plants showed severe chlorosis. The addition of iron counteracted this condition while the addition of small amounts of manganese intensified it. In the corresponding cultures in which distilled water was used toxicity was less severe.

TABLE 12

*Chlorosis and necrosis in bean plants after 18 days. Experiment 26-I*

Part "A"					Part "B"				
Mn	Fe				Cult. No.	Kind water	ppm		Chlorosis necrosis
	2 ppm	5 ppm	10 ppm	20 ppm			Fe	Mn	
<i>ppm</i>									
2	--	--	--	--	1	D.W.	0	0	--
5	++	--	--	--	2	T.W.	0	0	+-
10	++	+-	++	--	3	D.W.	5	0	--
20	++	++	++	+-	4	D.W.	0	$\frac{1}{2}$	++
					5	D.W.	5	$\frac{1}{2}$	--
					6	T.W.	5	0	--
					7	T.W.	0	$\frac{1}{2}$	++
					8	T.W.	5	$\frac{1}{2}$	--

One "plus" sign indicates chlorosis. Two "plus" signs indicate both chlorosis and necrosis.

TABLE 13

*Oven-dry weights after 31 days. Experiment 26-I. Part "B"\**

Cult. no.	Kind water	ppm		Dry weights		Total
		Fe	Mn	Tops	Roots	
				<i>grams</i>	<i>grams</i>	
1	D.W.	0	0	3.6	2.5	6.1
2	T.W.	0	0	3.2	1.6	4.8
3	D.W.	5	0	4.2	2.1	6.3
4	D.W.	0	$\frac{1}{2}$	1.7	1.7	3.4
5	D.W.	5	$\frac{1}{2}$	5.5	1.6	7.1
6	T.W.	5	0	5.5	3.5	9.0
7	T.W.	0	$\frac{1}{2}$	2.6	1.7	4.3
8	T.W.	5	$\frac{1}{2}$	5.7	2.7	8.4

\* Due to heavy production of root nodules in a number of the cultures of Part "A" the dry weights for that part are not given in full. At 20 ppm Mn the dry weights were:

	Fe ppm			
	2	5	10	20
Dry weight tops (Grams).....	1.2	3.0	2.9	5.4
Roots.....	0.9	4.1	2.6	3.5
Total.....	2.1	7.1	5.5	8.9

In both cases tomato plants were more severely affected than bean plants. This was particularly shown by plants in solutions prepared with distilled water and lacking both iron and manganese. They developed chlorosis in 14 days from planting while the bean plants in the corresponding cultures in Experiment 26-I remained green for about 20 days. While tomato plants may be actually more sensitive to manganese toxicity it is thought that a logical explanation of this is that the smaller reserves of iron in the smaller tomato seed are insufficient to balance the manganese impurities.

TABLE 14

*Effect of small traces of manganese in the tap water used in preparing culture solution on chlorosis in tomato plants. Experiment 26-K*

Cult. No.	Nutrient Solution Prepared with:	ppm		Chlorosis		
		Fe	Mn	Six days	Ten days	Seventeen days
1	Distilled water	0	0	—	—	++
2	Tap water	0	0	+	++	+++
3	Distilled water	4	0	—	—	—
4	Distilled water	0	0.4	+	++	+++
5	Distilled water	4	0.4	—	—	—
6	Tap water	4	0	—	—	—
7	Tap water	0	0.4	++	+++	++++*
8	Tap water	4	0.4	—	—	—

\* Plant was dead at this time.

#### *Experiment 26-J. Tomato*

This set-up was like that of Experiment 26-H with the following exceptions: (1) tomato plants were used, one to each of the 42 large culture vessels; (2) concentrations of manganese up to 1 ppm only were employed; (3) each series at the three manganese concentrations were in duplicate; and (4) a "zero" iron concentration was not used.

Tomato seeds of the variety "Marglobe" were shown in quartz sand which had been previously sterilized with hot water and then watered with a nutrient solution (macro elements only). The plants were about 2 inches in height when they were transferred to the cultures. The solution described on page 52 was employed and the cultures were randomized as before.

In the early stages practically all plants developed some degree of chlorosis, and at 6 days many of them showed definite patterns on the first pair of true leaves while the cotyledons in every case were entirely green. This is undoubtedly due to iron reserves in the cotyledons. A series of



observations and records were made of the extent of chlorosis, and while marked differences were found between the high iron cultures as contrasted with the high manganese cultures, such a definite conformity to treatment in the early stages was not found as was true for beans. However, at 23 days after planting and thereafter plants at high iron concentrations had recovered from the initial chlorosis while the others did not. Many of

TABLE 15

*Chlorosis and necrosis of tomato plants at 26 days. + = Chlorosis, D = Dead plants or those that will die. Experiment 26-J*

Mn	Fe						
	$\frac{1}{2}$ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
ppm							
0	+	+	-	-	-	-	-
0	+	+	+ D	-	-	-	-
$\frac{1}{2}$	+ D	+ D	-	-	-	-	-
$\frac{1}{2}$	+	+ D	+	-	+	+	-
1	+ D	+	+ D	+	+ D	+	-
1	+ D	+ D	+ D	+ D	+	-	-



FIG. 13. Antidoting effect of iron on manganese toxicity in tomatoes. Left—One ppm manganese and 4 ppm iron. Right—One ppm manganese and 8 ppm iron.

the latter showed various degrees of necrosis and quite a few were dead 23 days after planting. This is brought out in table 15 and also in figure 13 which is a photograph of two plants, both of which received 1 ppm manganese but different amounts of iron.

After 30 days the height of each plant from the base of the stem to the point where the topmost leaves originated was measured. These measure-

ments which are presented in table 16 show with some minor variations an excellent correlation with the iron manganese treatments. The general effect is also brought out in figure 14 where the values are plotted as a solid

TABLE 16

*Height of tomato plants in inches at 39 days. Experiment 26-J*

Mn	Fe						
	$\frac{1}{2}$ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
<i>ppm</i>							
0	3.1	3.4	7.7	8.1	9.1	9.1	15.4
0	4.5	2.4	6.1	5.1	6.6	9.5	—
$\frac{1}{2}$	3.5	4.0	—	5.4	9.2	5.5	6.1
$\frac{1}{2}$	2.6	2.6	2.2	7.4	4.6	8.0	9.1
1	2.9	2.0	2.5	3.1	1.7	4.5	2.5
1	2.6	2.6	1.2	2.1	2.9	3.9	6.2

TABLE 17

*Number of flower buds and flowers at 44 days. Experiment 26-J*

Mn	Fe						
	$\frac{1}{2}$ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
<i>ppm</i>							
0	0	0	6	8	13	10	10
0	4	0	6	4	6	9	—
$\frac{1}{2}$	0	0	0	4	5	4	2
$\frac{1}{2}$	0	0	0	0	4	7	6
1	0	0	0	0	0	6	6
1	0	0	0	0	0	0	0

TABLE 18

*Number of tomato fruits at 64 days. Experiment 26-J*

Mn	Fe						
	$\frac{1}{2}$ ppm	1 ppm	2 ppm	4 ppm	5 ppm	6 ppm	8 ppm
<i>ppm</i>							
0	0	0	5	6	6	5	10
0	0	0	3	2	5	6	—
$\frac{1}{2}$	0	0	—	1	3	4	4
$\frac{1}{2}$	0	0	0	5	2	7	0
1	0	0	0	0	0	2	6
1	0	0	0	0	0	2	2

figure. The same sort of relationship, as shown for the dry weights of bean plants, (figure 8) is also evident here.

A count of the number of flower buds and flowers at 44 days is given in table 17 and the number of fruits at 64 days in table 18. These also show a

marked correlation with treatment. A summary of the various observations follows in which the 21 cultures at high iron and low manganese are compared with the 21 at high manganese and low iron, using totals or averages.

Summary. Experiment 26-J. Tomato:

Item	High-iron Low-manganese	Low-iron High-manganese
Chlorosis, 5 days. ....	89	143
Chlorosis, 12 days. ....	83	145
Chlorosis, 14 days. ....	76	150
Chlorosis, 16 days. ....	73	156
Chlorosis, 20 days. ....	86	186
Chlorosis, 26 days. ....	5	18
Chlorosis, 34 days. ....	4	19
Necrosis, 36 days. ....	1	10
Height of plants, 23 days, average in inches. ....	4.68	2.81 <sup>1</sup>
Height of plants, 30 days, average in inches. ....	7.41	2.85
Number flower buds 44 days. ....	93	4
Number flowers open 44 days. ....	20	0
Number fruits, 64 days. ....	77	4

Dry weights of the plants were not determined but it is obvious that sufficient data were obtained to show clearly the relative effect of the two elements. Aside from the greater sensitivity of the tomato plants to manganese toxicity, the same relationship is evident that was found for bean plants.

#### *Experiment 26-I. Bean*

Bean plants were grown on a larger scale using the gravel subirrigation culture equipment described under "Experimental Method." The culture solution of the macro elements which was made in 50-gallon lots had the following composition:

Salt	Grams per liter
$\text{KH}_2\text{PO}_4$ .....	0.16
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	0.26
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.26
$(\text{NH}_4)_2\text{SO}_4$ .....	0.048

Iron was added as potassium iron humate and manganese as the sulphate. The plan of the experiment was as shown in the following diagram:

Mn	Fe				
	0	2	4	5	6
	Gravel bed numbers				
ppm					
0	1	2	3	4	5
1	6	7	8	9	10

Four rows of beans of 12 "hills" each were planted 1 foot apart with the side rows 6 inches from the sides of the bed. This gave a total of 48 hills in each of the 10 gravel beds. The seeds were planted at a depth of 1 inch or about at the upper limit of the culture solution at the end of the pumping period. The plants made excellent growth in all ten cultures

TABLE 19

*Lengths of the first trifoliate leaves in row two in each bud in centimeters. Nine days. Experiment 26-I*

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
ppm					
0	3.06 ±.075	2.52 ±.063	2.71 ±.11	2.94 ±.061	3.85 ±.096
1	4.37 ±.090	3.85 ±.104	4.46 ±.100	3.74 ±.189	3.62 ±.140

and up to the eleventh day there were no obvious differences. No chlorosis was observed throughout the experiment.

Unfortunately, possibly due to slight admixture of limestone particles with the igneous rock gravel used in the beds, the pH value of the solutions in all cases rose from 7.1 to 7.4 by the eleventh day. Because of this, the iron was precipitated, or absorbed, to a large extent, on the calcium humate that formed. Therefore, the concentrations in respect to iron in the various tanks were rather uncertain. In spite of this drawback from the experimental standpoint the results are presented because, first, some rather significant facts are brought out, and second, because the plants made such excellent growth that the experiment may be of interest for that reason. There are also significant differences in yield and in other respects between plants that showed no differences in appearance such as chlorosis, etc. This should be of practical importance. The various counts and measurements are given in tables 19 to 23.

At 9 days there is an increase in the size of trifoliolate leaves with increasing iron from 2 ppm to 6 ppm (table 19) at zero manganese, while at 1 ppm

TABLE 20

*Percentage of second trifoliolate open leaves, eleven days. Total number of plants per bed counted varied from 85 to 96. Experiment 26-I*

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
<i>ppm</i>					
0	30.1	24.2	52.2	46.7	39.8
1	59.8	47.9	60.6	58.9	58.2

TABLE 21

*Height of plants in centimeters. Eleven days. Experiment 26-I*

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
<i>ppm</i>					
0	9.20 ± .63	9.38 ± .70	7.94 ± .56	8.28 ± .62	8.08 ± .55
1	8.24 ± .52	8.03 ± .41	9.08 ± .61	7.82 ± .63	8.32 ± .51

TABLE 22

*Number of leaves per bed showing sunscald. Eighteen days. Experiment 26-I*

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
<i>ppm</i>					
0	6	11	9	37	13
1	69	57	34	14	17

TABLE 23

*Total dry weight of plants per bed in grams. Thirty-one days. Experiment 26-I*

Mn	Fe				
	0 ppm	2 ppm	4 ppm	5 ppm	6 ppm
<i>ppm</i>					
0	262	276	276	277	282
1	209	206	250	232	225

manganese there is no definite trend with change in the iron concentration. The leaves, however, were significantly larger where 1 ppm manganese

was used. The percentage of second trifoliate open leaves at 11 days (table 20) showed no definite relation to the iron concentration at either manganese concentration but again there was a significant increase as the manganese was increased from zero to 1 ppm. Both of these measurements indicate a more rapid growth at 1 ppm manganese in the early stages although measurements of heights of the plants showed no significant difference.

Later, evidence of manganese injury was shown by susceptibility to sunscald at 18 days. The degree of sunscald was not severe and it did not appear to affect the luxuriant growth of the plants. Careful counts of the total number of leaves affected in each bed revealed that practically all the sunscald occurred in the cultures to which manganese had been added where it was inversely proportional to the iron concentration. The slight amounts at zero manganese showed no differences as the iron varied. (See table 22 and figure 15.)

Examination of the results of the determinations of dry weights of plants as given in table 23 show that while there was no difference in the appearance of the plants there was a significant reduction in growth due to the addition of 1 ppm manganese. Statistical analysis by the pairing method gave odds of 768 to 1 that the difference is not due to chance. The increase in yield when manganese is omitted is about 22 per cent. Although as seen from the data there was a general increase in dry weight with increase in the iron the trend is somewhat irregular. The fact that there was not a more marked relationship is due no doubt to the high pH and iron precipitation as previously noted.

#### *Experiment 15-5. Pineapple*

Forty-two cultures were prepared in the large culture jars. The variety "Smooth Cayenne" was used. One-half of these cultures were devoted to a study of other minor elements and will not be considered here. All cultures, however, were randomized according to the previous scheme. Each treatment was in triplicate as shown below.

Culture numbers	Parts per million		Notes
	Iron	Manganese	
1, 2, 3	0	0	No minor elements added. Iron only, added as $\text{FeSO}_4$ Manganese only, added as $\text{MnSO}_4$
4, 5, 6	5	0	
7, 8, 9	0	2	
28, 29, 30	0	5	Manganese as $\text{MnSO}_4$ , iron as potassium iron humate, copper 2 ppm and boron zinc and aluminum $\frac{1}{2}$ ppm each.
31, 32, 33	1	5	
34, 35, 36	3	5	
37, 38, 39	5	5	
40, 41, 42	10	5	

*Culture solution*

	<i>gms/liter</i>
$\text{KH}_2\text{PO}_4$ .....	0.132
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.410
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	0.472
$\text{NH}_4\text{NO}_3$ .....	0.126
$\text{K}_2\text{SO}_4$ .....	0.166

Distilled water was used. pH of solution 4.5.

Uniform healthy slips from "Smooth Cayenne" plants were used and no replanting was necessary. The roots showed active growth 8 days after

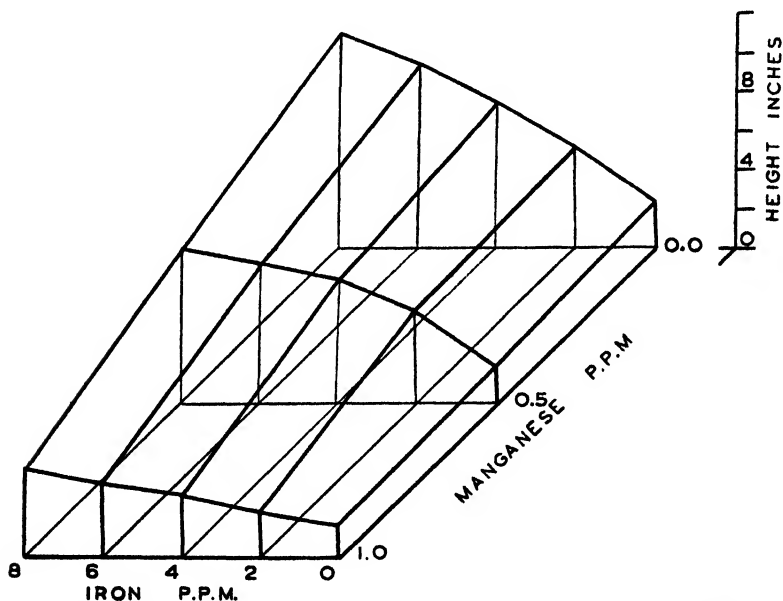


FIG. 14. Effect of iron and manganese on the growth of tomato plants. Experiment 28-J

planting and the plants all seemed to develop normally up to the 88 day. At this time slight but definite chlorosis was noted in cultures 28, 29 and 30 with 5 ppm manganese and no added iron. These cultures were severely affected by chlorosis at 97 days which was noted to increase in severity at 101 days. A photograph taken at 102 days (figure 16) shows culture 29 with no iron compared with culture 31 which in addition to the manganese had 1 ppm iron as potassium iron humate. The three cultures 28, 29 and 30 showed very clearly a variation in the time of appearance of manganese toxicity symptoms. Number 29 was the first to show them, then No. 30 and finally No. 28. This we consider to be due principally to

variation in the original slips in regard to the amounts of iron and manganese they contained. Later, necrosis showed up in the same order. The weights of the plants at 149 days also brings this out (No. 29, 518 gms; No. 30, 970 gms; No. 28, 1108 gms).

Meanwhile one of the three plants with 2 ppm manganese and no iron (No. 9) began to show chlorosis (94 days) and at 109 days No. 8 showed slight but definite chlorosis while No. 7, the third plant having the same treatment, showed no definite chlorosis at 143 days although the leaves

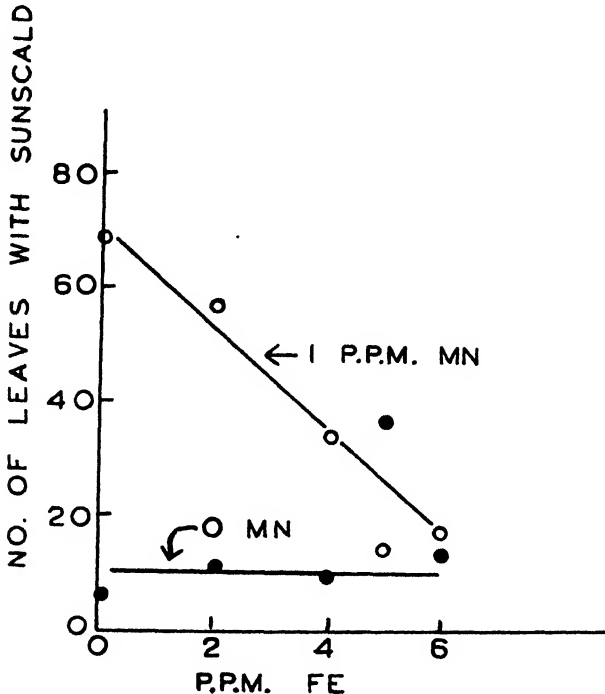


FIG. 15. Effect of iron and manganese on the susceptibility of bean plants to sunscald. Experiment 26-I

were not deep green in color. Here again the most severely affected plant was the first to develop necrosis. The plant had the lowest weight for this treatment (1218 gms). It had, however, a greater weight than culture No. 28 above.

The next treatment to show chlorosis in order of time and severity was that where all the minor elements were omitted: first, No. 2 showed slight chlorosis at 101 days and then No. 3 at 132 days. Necrosis was not observed in the case of any of these three cultures. All other cultures where iron was supplied developed no definite chlorosis although there was





FIG. 16. Antidoting effect of iron on manganese toxicity in pineapples. Above—Five ppm manganese and no iron. Below—Five ppm manganese and 1 ppm iron. Experiment 15-5.

some difference in the greenness of the leaves. Cultures 4, 5 and 6 which received 5 ppm iron in the inorganic form had the deepest green color. These plants, however, were definitely smaller with narrower leaves than those in treatments with certain combinations of iron and manganese.

These preliminary remarks are made to indicate the natural variation that occurs in the slips used for planting and to point out a possible explanation for this variation. They also indicate a method of evaluating the relative severity of manganese toxicity. Numerical values can be obtained by giving weight to: (1) the severity of chlorosis; (2) the number of plants affected; (3) time of the first appearance of chlorosis, and (4) the time of appearance and severity of necrosis. This has been done, and in lieu of giving detailed records of the many observations made, the weighted values for records up to 143 days are given below.

Treatment	Manganese toxicity weighted value
No iron, no manganese	4.5
Iron only, 5 ppm as $\text{FeSO}_4$	0.0
Manganese only, 2 ppm as $\text{MnSO}_4$	16.5
Manganese 5 ppm, no iron.	47.5
Manganese 5 ppm, iron 1 ppm as humate.	0.0
Manganese 5 ppm, iron 3 ppm as humate.	0.0
Manganese 5 ppm, iron 5 ppm as humate.	0.0
Manganese 5 ppm, iron 10 ppm as humate. . . .	0.0

The fact that some degree of manganese toxicity is evident where both iron and manganese are omitted, is brought about as previously discussed by manganese impurities. This differs from the result obtained by Schappelle (29, 30) where he found no chlorosis when all the minor elements were omitted from the solution. While there is some possibility that the difference may be varietal since he used "Red Spanish" pineapples, it appears more probable from the above discussion that a difference in the amounts of iron and manganese in the slips used will better explain the difference in the appearance of symptoms. It is seen also that as the manganese concentration increases to 5 ppm the severity of toxicity increases in the absence of iron. Iron in a concentration of 1 ppm is sufficient to antagonize this toxicity at least as far as visible symptoms are concerned.

After the plants had grown for a period of 149 days they were carefully removed from the cultures, and after blotting most of the free water from the roots they were weighed. The plant in each case was then quickly returned to its culture vessel. The results of the weighings are given in table 24. While there is a high probable error for the mean of any given treatment several points seem clear. Pineapple plants may be affected

to quite a degree by manganese toxicity and still show a greater fresh weight than others which show no toxicity symptoms. Compare treatments 1 and 3 with 2. Only when the toxicity is extremely severe is the fresh weight reduced. While iron at 1 ppm prevents chlorosis and death of the plants, at 5 ppm manganese the fresh weights are not significantly different. Increase in the iron content to 3 ppm and higher at this same manganese concentration brings about a significant increase in the fresh weight over zero and 1 ppm iron. The data given in table 24, therefore, should be studied in conjunction with the toxicity symptoms previously noted. The average height of the plants, also shown in table 24, are similar to the fresh weights in their relation to treatment.

The first fruiting occurred in culture No. 40 at 5 ppm manganese and 10 ppm iron. The fruiting stalk appeared at 210 days; the fruit developed

TABLE 24

*Fresh weights of pineapple plants after 149 days. Average height of plants, 215 days*

No.	Treatment	Fresh weight in grams				Average height  inches
		A	B	C	Ave.	
1	No Fe, no Mn	1592	1306	1315	1397	20.9
2	Fe, 5 ppm	998	1060	691	983	21.6
3	Mn, 2 ppm	1275	1718	1218	1409	23.2
4	Mn, 5 ppm	1108	518	970	863	14.5
5	Mn, 5 ppm; Fe, 1 ppm	930	714	842	826	23.7
6	Mn, 5 ppm; Fe, 3 ppm	1244	776	1633	1216	23.8
7	Mn, 5 ppm; Fe, 5 ppm	1134	1043	1050	1076	24.9
8	Mn, 5 ppm; Fe, 10 ppm	1438	706	1156	1107	25.2

normally and was harvested about 10½ months from planting when it was beginning to turn color near the base. Records were made in each case of the time of flowering and the time for maturity of the fruit. As the fruits matured they were harvested, weighed, measured and the juice analyzed. These data are presented in table 25.

At 5 ppm manganese it will be seen that the plants required the longest time to flower and mature fruit, in fact, one plant never flowered. As the iron content increased the time to flower and mature fruit became less. In this respect cultures without minor elements, those with iron alone, those with manganese alone at 2 ppm, and those with all the minor elements did not appear to differ significantly from each other. However, they required a longer time for flowering than those at the higher iron concentrations in the iron-manganese series. Likewise, at 5 ppm manganese, the total weight of the fruit (including the crown) was lowest and increased with the iron content. This is shown graphically in figure 17.

TABLE 25

*Effect of iron and manganese on the production of pineapple fruits. Experiment 15-5*

Culture numbers	Treatment	Days to flower, average	Days to mature fruit, average	Weight fruit-crown, average	Weight crown average	Total fruit & crown, average	Average diameter	Average height	Juice analysis		
									Invert sugar, 100 cc.	Total sugar, 100 cc.	Brix
				grams	grams	grams	inches	inches	grams	grams	degrees
1, 2, 3	-M.E.	424	536	965	395	1360	4.6	5.0	3.30	13.86	15.7
4, 5, 6	Fe 5**	423	534	1088	274	1362	5.1	4.9	3.10	11.26	13.2
7, 8, 9	Mn 2**	423	539	1057	261	1318	4.8	4.7	3.65	11.09	14.7
22, 23, 24	M.E.	411	533	1002	199	1201	4.6	5.1	4.86	13.28	14.8
28, 29, 30	Mn 5**	456*	675*	430	238	668	4.4	3.9	2.93	11.30	13.9
31, 32, 33	Mn 5 Fe 1**	415	525	1057	228	1285	4.6	5.5	5.72	10.55	12.6
34, 35, 36	Mn 5 Fe 3**	406	509	1064	319	1383	4.7	5.3	3.40	9.47	14.0
37, 38, 39	Mn 5 Fe 5**	406	514	1123	288	1411	5.2	6.2	3.66	11.80	13.9
40, 41, 42	Mn 5 Fe 10**	340	447	1078	376	1454	5.1	5.1	3.84	13.14	14.3

\* Two fruits.

\*\* Number following symbol for element indicates concentration in ppm.

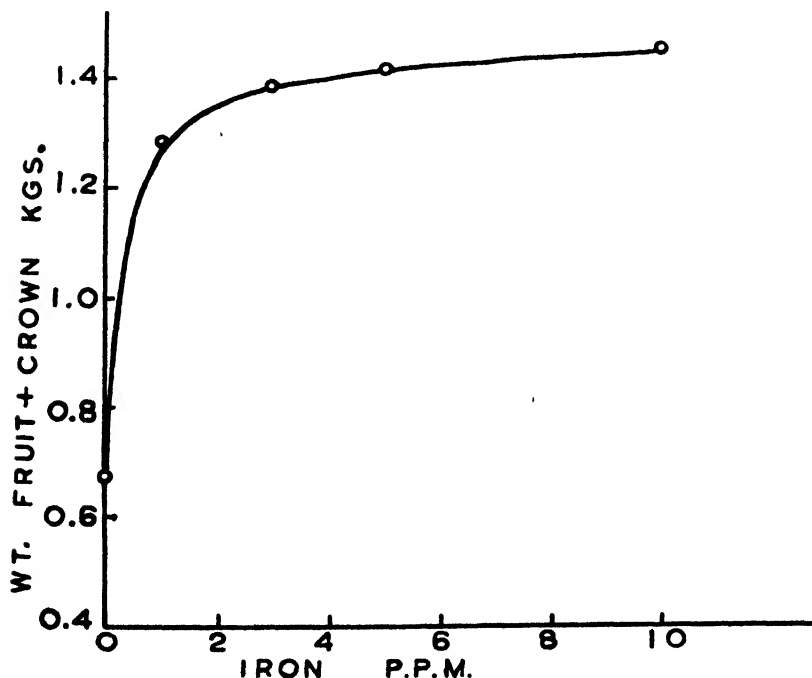


FIG. 17. Effect of iron on the production of pineapple fruits at 5 ppm manganese. Experiment 15-5

The large increase was from zero iron to 1 ppm iron. Without any minor elements the high proportion of the weight of crown leaves to that of fruit suggests a tendency towards a vegetative condition similar to that found by Schappelle (29, 30). The size of the fruit showed practically the same relationship to treatments as the weights. No particular correlation between the sugar content of the juice and the iron-manganese ratio is evident.

This experiment with pineapple plants shows the same antagonistic effect of iron on manganese as was shown by the experiments with beans and tomatoes. Certain differences due to the type of plant, the size of the seed piece, etc., are evident, but the antidoting action of iron is basically the same. The difference in time of appearance of the symptoms of manganese toxicity is of interest. Whereas with tomatoes and beans it is a matter of days, with pineapples it requires several months for chlorosis to show up. The effect of only 1 ppm iron in antidoting 5 ppm manganese as regards chlorosis, necrosis, height of the plants, and weights of fruit produced, is very striking.

*Experiments on the effect of light in relation to iron and manganese and the oxidation potential*

Light is an important factor in the action of iron and manganese on green plants. McCool (25) in 1913 found greater injury to plants from manganese in the light than in the dark although the latter were spindly and etiolated. He later reported (26) that with shading there was less injury from manganese in the form of chlorosis and necrosis although a decrease in dry weight was not prevented except in the case of tobacco. It was also noted by Gericke (5) that wheat plants in solutions devoid of iron remained green when grown in light of low intensity and produced excellent growth whereas in bright sunlight they made little growth and became markedly etiolated. Loewing (24) observed this same phenomenon. This latter case, according to the idea previously developed in this paper, can also be considered as manganese toxicity. The main point, however, is that light exerts a great effect on the symptoms of manganese toxicity or on iron deficiency. In this connection it was reported by Wann (36) that chlorosis of Concord grapes is most severe during periods of high light intensity.

We wish to present here the results of various observations and experiments made in the course of this work as well as some heretofore unpublished work by the senior author and attempt to point out their physiological significance.

"Short top" of pineapple fruits. That this symptom (see figure 2) is one of manganese toxicity is probable although the evidence is somewhat

circumstantial. It was observed to be very prevalent in Puerto Rico on soils with a high content of soluble manganese. On one plantation the following extraordinary condition was noted. In fields where the two row banks ran east to west "short top" was found almost exclusively in the south rows. A careful count showed a ratio of 25 to 2 of such fruits in the south row as compared with the north. In fields where the banks ran from north to south, short top fruits were evenly distributed throughout the field. Further, in one field with a high percentage of "short top" fruits none were found in one small area shaded by trees. On the contrary, the fruit in this area while somewhat smaller in size had abnormally long tops. A large number of leaves collected from "short top" plants showed no difference in their greenness from those from normal top plants. Chemical analyses of "short top" and normal fruit showed both to have a very high manganese content with no significant difference between the two. It was concluded that greater exposure to light brought out this particular symptom of manganese toxicity.

*Phototropism.* This is another effect of light in combination with manganese toxicity which can be looked on as manganese induced phototropism.<sup>7</sup> This interesting and striking phenomenon occurs in the seed leaves of bean plants grown in solutions with 20 ppm manganese and no added iron. (See figure 6.) As was described in Experiments 26-C and 26-F, in bright sunlight the seed leaves orient themselves so as to be parallel to the incident light. On cloudy days and at night they are horizontal. At the same manganese concentration 2 ppm of iron will prevent this phototropic movement.

*Sunscald.* In experiments reported in this paper it will be recalled that, where sunscald occurred, it was definitely associated with high manganese and low iron. Whether this is primarily a light or heat effect is uncertain. It is, however, a distinctly different condition than that of necrosis on plants at a toxic manganese concentration and not subjected to such extreme conditions of light and temperature.

*Photoreduction of iron as influenced by manganese.*<sup>8</sup> The tests to be described here were made *in vitro* with solutions but it is thought they may help to elucidate what may take place in the leaves of green plants. In the course of some experiments on plant nutrition a marked change in color was noted in one of two of the stock solutions: "A," which contained

<sup>7</sup> A similar movement of the leaves of *Glucidia sepium* in direct sunlight was described by Gates (4) and designated by him as "xerofotic" from the fact that it is due to differential turgidity in the pulvini caused by greater drying effect on the upper side under one-sided illumination. His observations, however, were made on apparently normal plants and no relation to nutritional factors was suggested.

<sup>8</sup> These observations and tests were made by the senior author at Cornell University and not previously published.

0.1 gm iron and 4 gms of sodium citrate and "B," which contained in addition to that in "A," 0.01 gm manganese. After these had stood in 100 cc volumetric glass stoppered flasks in a north window of the laboratory it was found that in "A" the greenish yellow color of the ferric citrate had disappeared. Solution "B," however, still retained the yellow color. This indicated reduction in "A" and not (or only partial reduction) in "B." On removing the stopper from "A" and agitating the solution the iron was reoxidized to the ferric condition with return of the yellow color.

This effect was investigated further in a series of test-tube experiments in which it was found that ferric citrate solutions like the above could be reduced to colorless in 5 to 10 minutes in direct sunlight (through window glass plus the glass of the containers for the solutions) and quickly reoxidized again by agitating them or bubbling air through the solutions. Methylene blue was used as an oxidation reduction indicator giving very striking color changes as the iron was reduced or oxidized. It was further found, as suggested by the above observation, that manganese had a retarding effect on the reduction of iron by light. Manganese also accelerated the reoxidation in the dark.

By placing a layer of oil above the solution to exclude atmospheric oxygen and exposing it to direct sunlight the solution could be reduced and prevented from reoxidizing in the dark. After a long time with slow diffusion of oxygen through the oil layer, slight oxidation would be noted at the top of the solution. Tests for ferric and ferrous ions were made by carrying out the reactions under oil. Solutions reduced in direct sunlight for 10 to 15 minutes gave no test for ferric ions with KSCN but a strong test for ferrous ions with  $K_3Fe(CN)_6$ . The reverse was true for solutions kept in the dark and in contact with air. As a further demonstration a photoelectric cell was prepared by connecting two solutions of iron citrate, one in the light and the other in the dark by means of an agar-KCl bridge and measuring its E.M.F.

The above experiments show: (1) that the oxidation-reduction of iron citrate (and probably other organic forms of iron) is reversible; (2) that light catalyzes the reduction of iron; (3) that manganese catalyzes the oxidation of iron; and (4) that the access of atmospheric oxygen is a factor in the rate of reduction or oxidation of iron.

*Discussion of the effect of light.* In applying these results to the leaves of green plants it is suggested that the state of oxidation of iron in leaves is by no means static but very changeable, depending on the light intensity, the permeability of the leaf tissues to light, the amount of manganese, the permeability of the tissues to oxygen and carbon dioxide, and the rate of photosynthesis and respiration. The latter processes would affect the composition of the intercellular atmosphere and thus affect the course of

oxidation and reduction. The organic composition of the leaf and its pH may be important factors in determining the solubility and ionization of the iron (Hopkins 11) and hence the rate of oxidation. The problem, because of the number of interrelated and, in nature, unpredictable factors is complex. Nevertheless, light appears to be of great importance in influencing the effect of iron and manganese on plant growth, and that its main rôle consists of its control of the oxidation potential.

Much more experimental work is needed to make clear the effect of the above factors, their interrelation, and mode of action, but for the present we suggest the following as a tentative hypothesis. Just as for factors such as pH and temperature, there is an optimum range of oxidation-reduction potential for the growth of green plants. Beyond these limits, either at a higher or lower potential, injury results. When manganese is present in high amounts in relation to iron the oxidation potential is above the optimum range and chlorosis followed by necrosis results, that is, symptoms of manganese toxicity (iron deficiency). Because of the lack of iron no system is present by which reduction can take place in the light and the oxidation process may even be accelerated under these conditions by light. This would explain previous results that manganese toxicity (iron deficiency) injury is greater in more intense light.

If cobalt is used in the absence of iron and the oxidation potential raised to a still higher level, as was done by Somers and Shive (33), toxicity is more severe than with manganese. With moderate amounts of iron and manganese in a ratio of, say, 2 to 1 or 10 to 1 an average optimum value or optimum range, depending somewhat on environmental conditions, is obtained and normal growth occurs. Lastly, if very low manganese and high iron concentrations are used the average potential falls below the optimum range and iron toxicity (manganese deficiency) results. In other words, without sufficient manganese there is no catalyst to oxidize the iron to the ferric condition. One, therefore, might expect the symptoms to be different from those of manganese toxicity as Somers and Shive found to be the case.

The above is based on the premise that iron, manganese and light are the principal factors in controlling the oxidation potential in the plant cell. However that may be, the experimental indications are that they do exercise a strong effect in this regard. This is an extension of the mechanism previously suggested by Hopkins (10) that manganese functions physiologically by its action on the state of oxidation of iron.<sup>9</sup>

<sup>9</sup> Other unpublished experiments by the senior author have shown that very little oxidation of hydroquinone takes place in the presence of iron without manganese or in the presence of manganese without iron but in the presence of both elements rapid oxidation occurs.



## DISCUSSION

It is clear from the data presented in this article that manganese toxicity is a problem in certain sections of Puerto Rico due to the development of toxic concentrations of soluble manganese in the soil. It is also true that this condition may occur in many other agricultural areas. The relation of iron in antagonizing or antidoting the effect of manganese has been shown to be of great value, and it is obvious that the manganese-iron relationship is not merely of laboratory interest but a practical matter. One thing that should be emphasized is that the balance between these elements should be carefully considered in what might be called the normal range. That is, manganese toxicity as exhibited by chlorosis and necrosis should, of course, be corrected, but a further study should be made to find the proper balance for highest crop yields.

Not forgetting that manganese is a necessary element for the growth of green plants (12) and is absolutely required in small amounts, it is of great practical value to know at what concentrations and under what conditions it becomes injurious. The factors that influence its toxicity should be known in order that the condition can be corrected.

Among these factors the relative concentration of iron and light intensity probably have the greatest influence. Attention in this work was directed to the iron factor, since it was more readily controlled than light. The antidoting effect of iron has been repeatedly shown both in soil and water culture tests. Where the concentrations of the two elements have been varied at the same time, a rather complete picture of their action is brought out and the relationship depicted graphically by 3-dimensional figures. This has shown that the effect produced on the plant is not due to one of the elements alone but by their interaction. Even at relatively low manganese concentrations marked toxicity may occur with insufficient iron and not at higher manganese concentrations with sufficient iron. This gives rise to an interesting anomaly since so-called "iron deficiency" symptoms can occur at a relatively high iron concentration and not at a lower one. As has been pointed out before this is due to the fact that iron at the higher concentration may be insufficient to balance the manganese, while at the lower concentration it may suffice. The matter becomes clear when iron deficiency is regarded as equivalent to manganese toxicity.

In attempting to elucidate the mechanism by which iron acts in antidoting manganese it can be postulated that its effectiveness depends on its being a reversible oxidation-reduction system which in the presence of certain proportions of manganese varies in a normal range of potential which green plants can withstand without injury. Within this range of

potential, iron can act as an oxygen donator or acceptor in carrying on the metabolic oxidations and reductions of the cell.<sup>10</sup>

Manganese acts as a catalyst in oxidizing iron and thus shifting the potential to a higher level. This is counterbalanced under normal conditions by the effect of light in reducing the iron and lowering the potential. With high manganese and low iron the manganese maintains too high a potential for normal growth and in the presence of insufficient iron light tends to intensify the toxic effect. Under such conditions the addition of iron may be looked on as a protective measure against high light intensity.<sup>11</sup> On the other hand, at high iron concentrations and low manganese, light reduces the iron and maintains a potential below the normal range. This causes iron toxicity. Iron toxicity was not found in the experiments reported here probably because in all cases the amount of manganese (from impurities) was sufficient to balance the highest amounts of iron used. It is hardly probable that iron toxicity is of any practical importance in Puerto Rico where iron is usually a limiting factor.

Important modifying effects on the action of iron and manganese taken up into the leaves of green plants are brought about by other factors such as pH of the cell sap,  $\text{PO}_4$  ion concentration, organic composition of the cell sap especially in respect to organic hydroxy acids or their salts, conditions more or less favorable for adsorption and the amounts of readily oxidized or reduced organic substances. These factors are practically all interrelated in their effect on the solubility, ionization and states of oxidation of iron. In other words they have a marked effect on the concentration of *physiologically active iron*.

Several of these factors are also concerned with the availability of iron and manganese in the substrata in which the plants grow. This is well known. Iron, for instance, may be precipitated at a high pH, and the effect is more pronounced at a higher  $\text{PO}_4$  ion concentration. Iron may become unavailable through adsorption, and this in turn is markedly affected by the pH, etc. Iron in organic combination is more soluble even at relatively high pH values but may be removed from such combination by adsorption.

<sup>10</sup> It is not intended here to place the whole matter of metabolism on such a simple basis as just outlined but merely to point out that an inorganic mechanism exists which can exert a strong effect in controlling the oxidation-reduction potential and the course of oxidations and reductions in the green plant.

<sup>11</sup> In this connection it would be of interest to know if such crops as coffee and vanilla, which usually require shade, could be grown successfully in strong light if furnished with a more abundant supply of iron. Hernández (The Journal of Agriculture, Univ. of Puerto Rico, 27: 27 1943) has shown that vanilla plants grown in full sunlight exhibit marked symptoms of toxicity and chlorosis.

In regard to the prevention of manganese toxicity where it occurs in Puerto Rico, final recommendations cannot be made until the results of field tests are available. However, several suggestions based on the findings presented in this paper can be offered. On acid soil with a high content of soluble manganese it is suggested that the manganese be immobilized by careful adjustment of the soil with finely ground limestone to pH 6.0. At least part of the fertilizer nitrogen should be in the form of nitrate to prevent the development of high acidity caused by the use of ammonium sulphate. The organic content of the soil should be increased to make the iron more available. In the case of pineapple culture iron sulphate sprays should be continued until the soil condition has been corrected.

While human and animal nutrition are beyond the scope of this article, the high manganese content of pineapple fruits analyzed in this work (see footnote page 46) suggests that both feed and foodstuffs produced under these conditions may contain excessive amounts of manganese. It is entirely possible that serious deleterious effects might result from long continued intake of relatively large quantities of this element. Another phase of this is the relation of manganese to vitamins. It acts as a catalyst in the acceleration of oxidations which are responsible for the destruction of the vitamins especially B<sub>1</sub> and C. As was also shown in this work by analysis of the tap water, that the water supplies in certain areas may contain relatively large amounts of manganese and be deficient in iron. Thus there is the possibility of toxicity due to excess manganese and lack of iron and also insufficiency of vitamins.

#### SUMMARY

Investigation of pineapple soils and pineapple plants growing on them have shown that severe conditions of manganese toxicity exist in Puerto Rico. While these soils do not have excessive amounts of total manganese, chemical analyses have revealed as high as 130 ppm of water soluble manganese and no water soluble iron. It is necessary to spray pineapple plants growing on these soils with iron sulphate solution to prevent severe chlorosis and death of the plants. This has become a common practice in Puerto Rico. Even when chlorosis is prevented with iron sprays large amounts of manganese are taken up as shown by analyses of the fruits and other peculiarities of the plants developed which appear to be associated with manganese toxicity. One of these known as "short top" shows an interesting relation to light intensity.

One cause of the development of high amounts of soluble manganese in the soil is the continued use of ammonium sulphate whereby the pH of the soil is often lowered to 4.0 or less. By careful adjustment of the

pH of the soil to 6.2 the manganese was immobilized, or made insoluble, to such an extent that the available iron was sufficient to antidote its toxicity as regards chlorosis. The addition of iron in organic combination was found to improve the condition still further.

A rapid method of detecting conditions in soils leading to manganese toxicity was used. This was done by growing common bean plants in the soil in question. Within a period of 10 days, when the first trifoliolate leaves opened, symptoms of chlorosis would appear and the approximate severity of the condition determined.

To study carefully the interaction of iron and manganese on the growth of plants extensive water cultures and subirrigation gravel culture experiments were carried out with beans, tomatoes and pineapples. The concentrations of these elements were usually varied simultaneously, iron being used in the form of potassium iron humate. Tomato plants were found to be more sensitive than beans to manganese toxicity and beans more sensitive than pineapples because of variation in the iron reserve in the seed or seed piece. For the same reason considerable variation in this respect was encountered in pineapple plants grown from different slips.

The relationship between iron and manganese in regard to growth is best shown by diagrammatic tables and 3-dimensional figures but the results can be expressed briefly as follows: Chlorosis, necrosis, sunscald and decreased growth of the plants were strikingly associated with low iron and high manganese, while such things as increased size and weight of the plants, earliness of appearance of trifoliolate leaves, tendrils, flowers, and fruits, and the rate of recovery from chlorosis were markedly associated with low manganese and high iron.

The ratios of dry weights of tops to roots indicate that the tops were affected more by variation in the iron-manganese relationship than the roots. In general the Fe/Mn ratio was found to be the controlling factor in growth, but for each given ratio growth varied with the total concentration of iron plus manganese.

An interesting effect of manganese on phototropic movements of seed leaves of bean plants was studied. At 20 ppm manganese, 2 ppm iron were sufficient to prevent these movements. This and other phenomena in respect to light have led to the idea that iron acts as a protective agent against light and also that the interaction of the 3 factors: iron, manganese and light, are important determinants controlling the oxidation potential of green plants. In proper balance a normal range of the oxidation potential results. When not in proper balance a too high or too low a range of potential for plant tissues occurs and toxicity appears.

Tentative recommendations are given for preventing manganese toxicity

and it is further suggested that much may be gained in the way of increased crop yields by careful adjustment of the iron-manganese balance within the normal range where symptoms of toxicity are not apparent.

#### ACKNOWLEDGEMENTS

The authors of this article wish to express their appreciation of helpful information, advice and assistance given them by various persons during the course of this work. Among the pineapple growers special thanks are due Mr. John Raymer of the Palo Blanco Fruit Company, Mr. Héctor Gandía of the Cidra Farm and Mr. Paul Zimmerman of the Palto Fruit Company for their many kindnesses and courtesies. Also of inestimable value was the help and advice of many members of the Staff of the Experiment Station. Their cooperation contributed much to the success of this investigation.

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## BIOGRAPHICAL SKETCH OF HARRY A. BEATTY

By OLIVER C. SKOV

Harry A. Beatty was born on St. Croix, January 10, 1903. He received his early training in natural science on his father's sugar cane plantation, an interest which matured with the years into a vocation. The first rudiments of an education were received by him from a private instructor and later he entered the Richmond High School, graduating in 1918. By that time he had brought together a sizable collection of insects, birds and eggs. In 1919 he attended the Mount Hermon School in Massachusetts, majoring in zoology. However, his studies at Mount Hermon were never completed, as he had to return home after his second year on account of financial difficulties.

Beatty's interest in natural history never lagged for a moment. In 1924 he was at Guánica, Puerto Rico, working in sugar cane cultivation, and he improved every opportunity for observation on the bird life of the region. It was during these ramblings that he made the acquaintance of Dr. Stuart T. Danforth who encouraged him to publish his notes on the birds of St. Croix, and later on the birds of Guánica Lagoon. He was sent to the Dominican Republic in 1925 where he made extensive observations on the bird life inhabiting the jungles and the savannahs around La Romana, and making frequent short excursions into the interior of that Island, when opportunity permitted.

He returned to St. Croix in 1929 and a few months later was in New York, studying medical entomology under a Professor of Medicine who was associated with Columbia University. He again returned to St. Croix in 1933, this time to enter the Department of Health as supervisor and biologist on a malaria and filaria research and control project. This involved extensive field and laboratory work with mosquitos, rearing and dissecting the infected insects.

Although preoccupied with these studies he always found time for investigations in other fields, and the discovery that several species of native birds harbored filaria parasites followed. Beatty's researches received the attention of Professor O'Connor, of Columbia University, and Professor Augustine, of Harvard Medical School; and in 1935 Dr. O'Connor spent the winter working with him, returning again in the summer of 1936 ac-



accompanied by Dr. Augustine. The results of these joint investigations were published as short papers in leading medical journals.

At the time little was known of the fresh water fishes and the mollusca, and extensive collecting was undertaken; the latter receiving special attention to determine their status among disease carriers. His interest in the birds of the islands received priority among his pet avocations. His paper on the birds of St. Croix was published in 1930, and later his notes on the birds of Guánica Lagoon appeared. Since then many interesting short papers in natural science, particularly in the Virgin Islands, have been published and there is promise of many others to follow.

In 1937 he went to Venezuela on the Weber expedition, collecting some birds, but he was particularly interested in searching for blood parasites. He made a collection of skeletal material for the United States National Museum. The following year saw him engaged in short excursions to St. Thomas and St. John, and for two summers he made observations on the pelagic breeding birds of the Virgin Islands, establishing new records and extending the range of several species.

In 1941 Beatty left the Department of Health to work with the Virgin Islands Wildlife Research and Restoration Project, a transfer which took him on a two-months study tour of several of the large game reservations in the United States. He held the post as supervisor and biologist of the project, until August 1944. Then Beatty went to Mona Island, remaining there for six weeks while making extensive collections of birds, insects, mollusca, and crustacea and some plants. In this journey he spent one week in the Luquillo Mountains of Puerto Rico searching for crows and parrots before returning to St. Croix to prepare his manuscripts for publication.

He is an enthusiast of the 16 mm. movie camera and his color films of birds and insects are always entertaining.

## INTRODUCTION

The island of St. Croix lies between 17th and 19th parallels north latitude and  $64^{\circ} 10'$  and  $65^{\circ} 30'$  west longitude. The length is 21 miles while the breadth is at most 6 miles on the western end, tapering wedgelike to a point on the eastern end. The mountain system, extending the length of the island, runs parallel to the north coast and fans out on the south side to form an extensive plain which, roughly, takes in about one third of the island. The highest peak is in Mount Eagle, near the centre of the range, at 1165 feet. The radiating spurs, abutting the shore line, come together to form valleys culminating in numerous bays.

On the south side the lowlands are planted to sugar cane and pasture lands. The western tip and eastern one third of the island are covered mainly with a semi-xerophytic type of vegetation, with dense thickets of organ cactus and bull cactus predominating. Of the original forests, which clothed the Island during an earlier era, only small pockets in the less accessible valleys have survived the onslaught, and there is some natural reforestation taking place where the second growth is reclaiming at a rapid rate large tracts of land which formerly were utilized for agricultural development.

The precipitation varies greatly from year to year, it being anywhere from 30 inches to 90 inches. Much of the rainfall finds its way into the sea but there are five quite large depressions along the coast, that become water holding areas, supporting a variety of bird and insect life. However, during drought years these so-called ponds turn into dust bowls. The few mangrove swamps on the Island are quite extensive.

The rainfall averages 46 inches annually, having a remarkable distribution over such a small land mass. The eastern one third receives approximately one half as much of the normal rainfall as the remainder of the Island and, therefore, has no permanent streams nor surface springs. The remaining portion of the Island is seamed with numerous streamways, although only a few of them maintain running water throughout the year.

The temperature is uniform, the daily and yearly fluctuations being gradual, and therefore, not marked. There is, however, a decided cooling off in temperature toward nightfall, which in the winter months can become quite cold. The average maximum on record is  $92^{\circ}$  and the minimum  $59^{\circ}$  with slight variations in either direction during some years. The humidity is not constant as there is not sufficient stored surface moisture to maintain it. During the dry seasons the evaporation rate is comparatively high, insects become inactive, and it would appear that there is no seasonal timetable for reproduction.

The short rain season comes in April-May, bringing with it the first burst of new foliage, and masses of flowers and winged insects begin swarming at dusk. The heavy rainfall occurs from October until January and many plants bloom a second time. Insects swarm again, following a rest period, and many species assume pest proportions.

The material upon which the present report is based was collected by the writer during the period from 1934 to 1945 and deposited in several institutions of scientific research. Much care was given to the preparation of the papers. The scientific nomenclature brought up-to-date in so far as has been made possible by existing limitations and although some slight discrepancies are sure to turn up, it is believed that on the whole, the publication of these papers without further delay should be undertaken; its value enhanced by the knowledge that no general treatise has appeared in print hitherto. It especially is in recognition of this latter circumstance that the task of accumulating the material was undertaken.

In the past there have been occasional short papers which discuss the various St. Croix faunas, but a relatively small portion of this literature is based upon study of extensive material, and, in fact, deals with odds and ends of insect material picked up by wandering naturalists. St. Croix seems always to be taken for granted and never seriously considered as an important integral part in any scientific survey of the Greater Antilles. To mammalogists it comes as a surprise to learn of the presence of a species of deer on the island, and that it occurs there abundantly. Ledru, in 1810, probably gave the earliest reference to the pelagic fauna, and in 1871 Cope discussed the ichthyology of St. Croix, but neither of these writers made mention of its fresh-water fishes.

The writer has already published on the "Birds of St. Croix" (Jour. Dept. Agr. P. R., 14(3): 135-150. Rio Piedras, August 1930), and, with my subsequent collections, this has been expanded by the late Dr. Stuart T. Danforth into a "Supplementary Account of the Birds of the Virgin Islands, with Notes on their Food Habits" together with a "List of Birds Known to Occur in the Virgin Islands" (Jour. Agr. U. P. R., 19(4): 439-72. Rio Piedras, December 1935). Chapman Grant in "The Growth of Herpetology in Puerto Rico and the Virgin Islands Area" (Jour. Dept. Agr. P. R., 16(4): 401-4. Rio Piedras, February 1933), lists all the reptiles from St. Croix.

# THE ENDOPARASITES OF ST. CROIX, V. I.

By HARRY A. BEATTY

St. Croix enjoys a phenomenal freedom from the innumerable types of endoparasites which prey upon the mammals of the world and for that reason a check-list is offered to give proper recognition of the prevalent forms. The various organisms came under the writer's observation and were studied during a period of research with the Department of Health from 1933 until 1941. The bulk of Cestoda was determined by H. L. Van Volkenberg and in each case the initials V. V. following the name is an indication of this.

## THE PROTOZOA

### SARCODINA (RHIZOPODA)

#### GYMNAMOEBIDA

1 **Endamoeba histolytica**

Intestines of man.

2 **Endamoeba coli**

Intestines of man.

### FLAGELLATA (MASTIGOPHORA)

#### MONOZOA

1 **Trypanosoma lewisi**

Blood parasite of rat *Rattus norvegicus*.

2 **Trichomonas humanis**

Intestines of man.

3 **Trichomonas** sp.

In ceca of fowl.

4 **Chilomastix mesnili**

Intestines of man.

#### DIPLOZOA

1 **Giardia lamblia**

Intestines of man.

### INFUSORIA (CILIATA)

#### HETEROTRICHIDA

1 **Balantidium coli**

Intestines of man and swine.

## SPOROZOA

## COCCIDIIDA

1 **Isospora hominis**

Intestines of man.

## HAEMOSPORIDIA

1 **Plasmodium vivax**

Man.

2 **Plasmodium malariae**

Man.

3 **Plasmodium falciparum**

Man.

4 **Haemoproteus** sp.

Observed in the nucleated red blood corpuscles in the white-crowned pigeon *Columba leucocephala*.

5 **Anaplasma marginale**

In blood of cattle.

## PLATYHELMINTHES

1 **Fasciola hepatica**

In cattle and sheep. Det. V. V.

2 **Postharmostomum gallinum**

Det. W. A. Hoffman. Eight specimens were recovered from the cecum of a turkey chick that was fourteen days old.

## CESTODA

1 **Dipylidium caninum**

Det. V. V. A common parasite in dogs.

2 **Hymenolepis nana**

From man.

3 **Taenia solium**

From man.

4 **Taenia saginata**

From man.

5 **Cystercercus tenuicollis**

This is not an uncommon worm in sheep but of many hundreds of wild deer that I have examined I found this worm in two instances only.

6 **Moniezia expansa**

Det. V. V. Found in sheep and goats.

7 **Anoplocephala perfoliata**

Det. V. V. Found in horse.

## NEMATHELMINTHES

## NEMATODA

- 1 **Strongyloides stercoralis**  
Intestines of man.
- 2 **Trichuris ovis**  
Det. V. V. In sheep.
- 3 **Trichuris vulpis**  
Det. V. V. In dogs.
- 4 **Trichuris trichiura**  
Intestines of man.
- 5 **Ancylostoma caninum**  
From dogs.
- 6 **Necator americanus**  
From man.
- 7 **Oesophagostomum radiatum**  
Det. V. V. From cattle.
- 8 **Oesophagostomum columbianum**  
Det. V. V. From sheep.
- 9 **Trichostrongylus colubriformis**  
Det. V. V. From sheep and goats.
- 10 **Strongylus vulgaris**  
Det. V. V. From horses and mules.
- 11 **Strongylus** sp.  
Det. V. V. From horses and mules.
- 12 **Bunostomum phlebotomum**  
Det. V. V. From cattle.
- 13 **Bunostomum trigonocephalum**  
Det. V. V. From sheep and goats.
- 14 **Crassisoma urosulatum**  
Det. V. V. From swine.
- 15 **Stephanurus dentatus**  
Det. V. V. From swine.
- 16 **Habronema muscae**  
Det. V. V. From horses and mules.
- 17 **Dictyocaulus viviparus**  
Det. V. V. From cattle.
- 18 **Heterakis gallinae**  
From domestic fowl.
- 19 **Enterobius vermicularis**  
From man.
- 20 **Oxyuris equi**  
Det. V. V. From horses and mules.

- 21 **Ascaris lumbricoides**  
From man.
- 22 **Ascaris equorum**  
Det. V. V. From horses and mules.
- 23 **Ascaris** sp.  
From rat *Rattus rattus alexandrinus*.
- 24 **Ascaris** sp.  
From dove *Zenaida aurita zenaida*.
- 25 **Ascaridia galli**  
Det. V. V. From turkey and fowl.
- 26 **Toxocara canis**  
Det. V. V. From dogs.
- 27 **Oxyspirura mansonii**  
From domestic fowls.
- 28 **Wuchereria bancrofti**  
Blood parasite of man.
- 29 **Vagrililaria columbigallinae**  
Blood parasite of ground dove (*Columbigallina passerina nigrirostris*).
- 30 **Filariidae** gen & sp.  
Blood parasite of ani (*Cratophaga ani*).
- 31 **Dirofilaria immitis**  
From dogs.
- 32 **Onchocerca cervicalis**  
Det. V. V. From cattle.
- 33 **Setaria equina**  
Det. V. V. From horses and mules.
- 34 **Trichinella spiralis**  
From swine.

## THE ARACHNIDA OF ST. CROIX, V. I.

By HARRY A. BEATTY

This check-list of the ectoparasites occurring on mammals, birds and reptiles of St. Croix, V. I., holds no pretense for completeness but the material at hand is sufficiently representative to gain our interest, and especially is this true since the presence of these parasites have remained unrecorded from the island. All species mentioned here were taken off of their natural hosts and the determinations were made by H. E. Ewing, excepting the half-engorged nymphs of *Amblyomma* which were determined by C. N. Smith. This being the first reference to *Amblyomma* on St. Croix it may have arrived here quite recently, possibly from Antigua, B.W.I. where it is reported as common.

To encompass a complete faunal report on St. Croix it should be mentioned here that a large collection of spiders was sent to Elizabeth B. Bryant and her paper entitled "Notes on the Spiders of the Virgin Islands" appeared in the "Bulletin of the Museum of Comparative Zoology," Vol. LXXXIX, No. 7, 1942.

### Order ACARINA

#### Suborder ASTIGMATA

##### SARCOPTIDAE

- 1 **Notoedres cati** Hering  
Off domestic cat.
- 2 **Cnemidocoptes** sp.  
Known as "scaly legs" of fowls.
- 3 **Sarcoptes scabiei canis** Gerlach  
Off dog.
- 4 **Psoroptes communis** Furstenberg  
Taken off cattle.

##### ANALGESIDAE

- 1 **Analgesidae** gen & sp.  
Off Red-tailed Hawk (*Buteo jamaicensis*), and Bare-legged Owl (*Otus nudipes newtoni*).
- 2 **Analgesidae** gen & sp.  
Taken off Ani (*Crotophaga ani*), Mocker (*Mimus polyglottos orpheus*), Thrasher (*Margarops f. fuscatus*), Yellow warbler (*Dendroica petechia cruciana*).



3 **Analgesidae** gen & sp.

Taken off Oyster-catcher (*Haematopus ostralegus palliatus*).

4 **Analgesidae** gen & sp.

Taken off Ground Dove (*Columbigallina passerina nigristrois*),  
Hummingbird (*Sericotes h. holosericeus*), Kingbird (*Tyrannus d. dominicensis*).

5 **Eupterolichus** sp.

Off Ani (*Crotophaga ani*).

6 **Falculifer** sp.

Off Scaled Pigeon (*Columba squamosa*).

7 **Pterodectes** sp.

Off Mocker (*Mimus polyglottus orpheus*).

## Suborder BRACHYPODA

## DEMODICIDAE

1 **Demodex canis** Leydig

Taken off dog.

## CHEYLETIDAE

1 **Cheyletus** sp.

Off domestic fowl.

## Suborder MESOSTIGMATA

## DERMANYSSIDAE

1 **Liponyssus bursa** Berlese

Off domestic fowl.

## PARASITIDAE

1 **Parasitidae** gen & sp.

Taken off a beetle, *Metamasius hemipterus*.

2 **Echinolaelaps echidninus** Berlese

Off gray Rat, *Rattus rattus alexandrinus*.

3 **Periglischrus** sp.

Off leaf-nosed bat, *Artibeus j. jamaicensis*.

4 **Gekobia** sp.

Taken from the abdomen of a gecko, *Hemidactylus mabouia*

5 **Macrocheles** sp.

Clustered about the thorax of beetle, *Cerambycidae* sp.

6 **Galumna** sp.

Collected from an old bird nest.

## IXODIDAE

1 **Ixodidae** gen & sp.

Taken off Bat, *Noctilio leporinus mastivus*. Taken off Mongoose, *Herpestes birmanicus*.

2 **Haemaphysalis chordeiles** Packard

Taken off Wilson Plover (*Charadrius wilsonia*).

3 **Dermacentor nitens** Neumann

A common parasite of the Horse (*Equus caballus*) and Donkey (*Equus asinus*). Rarely found on the deer (*Odocoileus virginianus*).

4 **Rhipicephalus sanguineus** Latreille

Common parasite of dogs.

5 **Boophilus annulatus microplus** Canestrini

Abundant parasite of deer (*Odocoileus virginianus*), and cattle.  
Rare on sheep and goats.

6 **Amblyomma** sp.

Det. C. N. Smith. Nymph taken off man. May 1935.

## THE INSECTS OF ST. CROIX, V. I.

By HARRY A. BEATTY

A study of Orthoptera and Isoptera have considerable interest, not only in the presence of several undescribed forms, but especially in the absence of species otherwise quite common on neighboring islands. Lepidoptera has received scant attention from entomologists. W. P. Comstock records 33 forms of the suborder Rhopalocera in "Scientific Survey of Puerto Rico and Virgin Islands," Vol. XII, part 4. My collection adds 10 forms, most of them common, to his list, and questions the occurrence of *Urbanus dorantes cramptoni* on St. Croix as no specimens have been taken, while *U. proteus* is an extremely abundant form. The status and economic value of the parasitic Hymenoptera is badly in need of determination. Some field work has been revealing and several very interesting observations would indicate that parasitization of many forms, in the Orthoptera, Diptera and Lepidoptera for example, apparently maintain them at a low status that is of economic importance. The absence of fruit infecting species of Diptera is quite marked. There are no records of occurrence of fruit-flies, *Anastrepha* species, in nispero (*Sapota achras*), papaya (*Carica papaya*), guavas (*Psidium guajava*).

The capture of six specimens of a Reduviid of the *Triotoma* family is of importance, since it established the presence of a genus that is well known to medical science as the host to *Trypanosoma*, which is a blood parasite of man. A great amount of time was devoted to research on Diptera, in particular the *Culicidae* and certain other species which infest flesh wounds. So far, *Cochliomyia americana* has not been taken on St. Croix. The study of *Culicidae* centered around a malaria and filaria control project sponsored by the Department of Health. The writer was in charge of the work for eight years and considerable laboratory and clinical experimentation was probed.

The writer devised a net which was extremely successful in capturing flying insects at dusk. By its use, many rare things were taken, and it will, no doubt, prove itself in other presumably well collected islands. The thing is simply a strong wire frame three feet long and one foot wide, fitted with a baggy fine-mesh netting, attached securely on the back of the seat and extended about one foot above the chauffeur's head on a topless roadster, also minus a windshield. Moving along at from five to ten miles per hour, at every half mile a stop was called and the catch collected, using a flash-light and aspirator; for speed is required to capture the active insects such as Staphylinidae.

THYSANURA

Determinations by Ashley B. Gurney

1 *Nicoletia* sp.

Taken in rotting log, Jealousy streamway. April 1938.

2 *Lepismatidae*. Genus?

"Clearly a different genus to *Nicoletia* because of head shape and the presence of eyes." Taken in rotting stump, Jealousy streamway. April 1938.

3 *Lepismatidae*

Probably *Nicoletia* sp. "A somewhat unusual Thysanuran and apparently belongs to *Nicoletia*."

ORTHOPTERA

The following records are based on material determined by Ashley B. Gurney, and deposited in the U. S. National Museum.

FORFICULIDAE

1 *Doru albipes* F.

Under decomposing vegetable matter. May 1938.

2 *Labia curvicauda* Motschler

Taken at light. January 1936. Under decomposing vegetable matter. October 1936.

LABIDURIDAE

1 *Euborellia plebeja* Dohrn

Under rubbish heap. January 1939.

BLATTIDAE

1 *Aglaopteryx* n. sp.

Common in rotting stumps and under dry-scaling bark, in mats of dry vegetation draping over branches. May 1936.

2 *Supella supellectilum* Serville

A common species, at night on flowers of *Calyptrocordia alba*. October 1936. Attracted by light to houses. February, November, December, 1937. Under rubbish heaps. In sugar cane straw. March 1938.

3 *Cariblatta antiguensis* S. & Z.

Common under heaps of rubbish. Attracted by light to houses. September 1936. On fruit of *Calyptrocordia alba* and on leaves of *Terminalia catappa*. May & October, 1938.

- 4 **Ichnoptera rufa** DeGeer  
Common under rubbish and on shrubbery at night. On fruit of *Calyptrocordia alba*. April 1938.
- 5 **Symploce bilabiata** R. & H.  
Common. Attracted by light to houses. Under rubbish. On shrubbery at night. On fruit of *Calyptrocordia alba*. May and November, 1938.
- 6 **Symploce hospes** Perkins  
Common. Under rubbish. On shrubbery at night. Attracted by light to houses. On fruit of *Calyptrocordia alba*. May 1938.
- 7 **Symploce** n. sp.  
Uncommon. Under rubbish and on shrubbery at night. May 1938.
- 8 **Periplaneta americana** Linn.  
Very common in buildings. Ootheca parasitized by *Tetrastichus hagenowi*. 1935.
- 9 **Periplaneta brunnea** Burm.  
Uncommon. In buildings. 1935.
- 10 **Periplaneta australasiae** F.  
A common species in sugar cane fields and in woodlands, uncommon in buildings. 1935.
- 11 **Eurycotis improcera** Rehn  
Common in rotting stumps, under scaling bark. 1935.
- 12 **Leucophaea maderae** F.  
Not common. Local, in buildings. Feed on meat scraps and fruits. The ootheca is retained within the body of the female and the living nymphs extruded. 1935.
- 13 **Pycnoscelus surinamensis** Linn.  
Common under rubbish. Frequently seen around chicken roosts feeding on droppings. Is host to the eye-worm, *Oxyspirura mansonii*.
- 14 **Hemiblabera** n. sp.  
This species is confined to the arid, eastern end of the island where small colonies can be turned up under heaps of rubbish. Not common. Specimens taken at Tagus. 1935.
- 15 **Plectoptera infulata** R. & H.  
Rare. Only five specimens taken from foliage of *Lantana involucrata*. Constitution Hill. February 1939.
- 16 **Holocampsa nitidula** F.  
Rare. Only three specimens were taken from a cellar, Mt. Pleasant. January 1941. One specimen collected from a shrub, June 1941, at River.

## MANTIDAE

1 **Callimantis** sp.

Specimens were taken on foliage of *Lantana involucrata*. Barren Spot. May 1937. Rare.

## PHASMIDAE

1 **Clonistria** sp.

Sept. 1937.

## ACRIDIDAE (LOCUSTIDAE)

1 **Schistocerca americana** Drury

An uncommon locust. 1935.

2 **Schistocerca columbina** Thunberg

Uncommon. 1935.

3 **Scyllina (Plectrotettix) gregarius** Saussure

1935.

## TETTIGONIIDAE (LOCUSTIDAE)

1 **Neoconocephalus triops macropterus** Redt.

Common. September to Jan. 1935.

2 **Neoconocephalus triops fuscostriatus** Redt.

Less common. 1935.

3 **Heterocous** sp. allied to *dubius* Caudell

In rotting logs. November 1935.

## GRYLLIDAE

1 **Scapteriscus vicinus** Seuddler

Rare, four specimens were collected in backyard soil in Christiansted where fruits imported from Porto Rico are distributed and the species may have been brought in. 1939.

2 **Anurogryllus muticus** DeG.

Common. July 1937.

3 **Gryllus assimilis** Fabr.

Uncommon. July 1937.

4 **Orocharis** sp.

Uncommon. In rotting branches. October 1937.

5 **Orocharis** sp. near *saltator* Uhler

Rotting wood. October 1937.

6 **Amphiacusta caraihea** Saussure

Common. October 1937.

7 **Oecanthus niveus** DeG.

Common on weeds. November 1935.

## ISOPTERA

The writer is grateful to T. E. Snyder and H. R. Johnston for determination of all species of termites. There seems to be no account given in the literature on the manner in which termite colonies go about enlarging their nests. I have watched this procedure on several occasions and give here a brief history. The work is done at night. Tiny holes one sixteenth of an inch and about one inch apart are cut through the outer shell of the nest and from these apertures a greater part of the entire colony of soldiers and workers emerge. At once the workers trail off in several directions in columns sometimes five and six abreast and so closely packed as to be treading on each other's heels. Once these trails are established the workers may be observed traveling in both directions; the heavy laden returning to the nest with material or supplies. At the nest construction goes ahead at an energetic pace. Before daylight is heralded each tiny hole has a roof and the nest is at least one cell larger in diameter.

## KALOTERMITIDAE

1 *Kalotermes* (K) *bequaerti* Snyder

In a rotting log, near Crique streamway. April 1938. Det. H. R. J.

2 *Kalotermes* (K) *snyderi* Light

A small colony in a dry-rotting log, winged adults, Prosperity Garden. April 1938. Det. H. R. J.

3 *Kalotermes* (K) *incisus* Silv.

Workers and soldiers from a decaying log, Crique. September 1938. In a dry branch on the ground, Estate Fountain. January 1939. Det. H. R. J.

4 *Kalotermes* (*Cryptotermes*) *cavifrons* Banks

A small colony in a decaying mango stump (*Mangifera indica*), La Grange. March 1938. Det. H. R. J.

5 *Kalotermes* (*Cryptotermes*) *brevis* Walker

Winged adults taken at light. May 1938. Small colonies in woodwork and furniture. Det. T. E. S.

6 *Kalotermes* (*Cryptotermes*) sp.

Taken from rotting stump of *Hippomane mancinella*, South Gate Farm beach. April 1935. Det. T. E. S.

## RHINOTERMITIDAE

1 *Heterotermes convexinotatus* Snyder

In a dry-rot pine board, tunnels on soil, Coakley Bay (semi-arid region). May 1938. Some workers and soldiers taken from be-

neath dry cattle droppings, Barren Spot. September 1940. Det. H. R. J.

2 **Heterotermes** sp.

Taken from a rotting stump, Prosperity Garden March 1938. Det. T. E. S.

TERMITIDAE

1 **Nasutitermes** (N) **acajutlae** Holmgren

Nest on *Hippomane mancinella*, Shoys, April 1938. In dry branch of *Spondias mombin*, Estate Collins, March 1938. Rotting stump of *Coccoloba wifera*, Tagus. March 1938. Also taken on Green Cay Islet. Det. H. R. J.

2 **Nasutitermes** (N) **costalis** Holmgren

A small nest placed in a small tree. Three queens, macropterous and physogastric, were removed from a queen-cell. Crique. April 1938. Det. H. R. J.

3 **Termes** (T) **panamaensis** Snyder

Winged adults were taken at light. Constitution Hill. June 1938. Det. H. R. J.

4 **Mirotermes** n. sp.

Winged adults taken at light. Western suburbs of Christiansted. May 1935. Det. T. E. S.

EMBIDINA

OLIGOTOMIDAE

Determinations were made by Ashley B. Gurney. Specimens in the U.S.N.M.

1 **Oligotoma saundersii** Westwood (= *O. latreillei* Rambur)

Taken under rubbish near Lower Love streamway. October 1940.

Taken at light. Golden Grove. October 1939.

2 **Oligotoma** sp. taken at light, Golden Grove. October 1940.

OLIGEMBIIDAE

1 **Oligembia brevicauda** Ross

Taken under rubbish near Castle Burke streamway. August 1940. Endemic, TYPE "under rubbish", Lower Love, August 1940.

CORRODENTIA

Determinations by Ashley B. Gurney.



## PSOCIDAE

- 1 *Lachesilla pedicularia* L.
- 2 *Peripsocus* sp.

## MALLOPHAGA

## PHILOPTERIDAE

- 1 **Philopteridae** gen & sp.  
Taken off ground dove (*Columbigallina passerina nigristrois*), thrasher (*Margarops f. fuscatus*), kingbird (*Tyrannus d. dominicensis*).
- 2 **Lipeurus gallipavonis** Geoffroy  
Taken off turkey.
- 3 **Lipeurus tropicalis** Peters  
Off domestic fowl.
- 4 **Lipeurus** sp.  
Off thrasher (*Margarops f. fuscatus*).
- 5 **Goniodes dissimilis** Nitzsch  
Taken off domestic fowls.
- 6 **Goniodes meliagris** Linn.  
Off domestic turkey.
- 7 **Goniodes numidae** Mjoberg  
Taken off guinea-fowl (*Numida meleagris galeata*).
- 8 **Esthioterum crassicorne** Scopoli  
Taken off the Bahama pintail (*Anas bahamensis*).
- 9 **Columbicola** sp.  
Off ground dove (*Columbigallina passerina nigristrois*), scaled pigeon (*Columba squamosa*).
- 10 **Rallicola bisetosa** Piaget  
Off clapper rail (*Rallus longirostris caribacus*).
- 11 **Philopter** sp.  
Off the ani (*Crotophaga ani*), thrasher (*Margarops f. fuscatus*).
- 12 **Trichloectes** sp.  
Off hummingbird (*Sericotes h. holosericeus*).
- 13 **Physconelloides zenaidurae** McGr.  
Off ground dove (*Columbigallina passerina nigristrois*), scaled pigeon (*Columba squamosa*).
- 14 **Proctophylloides** sp.  
Off hummingbird (*Sericotes h. holosericeus*)

## TRICHODECTIDAE

- 1 **Felicola subrostrata** Nitzsch  
Taken off cat.

## MENOPONIDAE

- 1 **Menopon gallinae** Linn.  
Off domestic fowl.
- 2 **Menopon numidae** Giebel  
Off guinea-fowl (*Numida melcagris galeata*).
- 3 **Eomenacanthus stramineus** Nitzsch  
Parasite on domestic fowls.
- 4 **Menacanthus** sp.  
Taken off ground dove (*Columbigallina passerina nigrirostris*).
- 5 **Myrsidea incerta** Kellogg  
Off thrasher (*Margarops f. fuscatus*).
- 6 **Trinoton querquedulae** Linn.  
Off Bahama pintail (*Anas bahamensis*).
- 7 **Neocolpocephalum flavescens** Nitzsch  
Off red-tailed hawk (*Buteo j. jamaicensis*).
- 8 **Colpocephalum** sp.  
Off ani (*Crotophaga ani*).
- 9 **Colpocephalum** sp.  
Off domestic turkey.
- 10 **Heterodoxus longitarsus** Piaget  
Occasionally found on dogs. Det. J. C. Bequaert.

## GYROPIDAE

- 1 **Gyropus ovalis** Nitzsch  
Off guinea-pig (*Cavia cobaya*).

## ODONATA

The dragonfly material which has served as a basis for the present paper was brought together over a period of years. Finding and rearing nymphs was one feature that received much attention but did not yield any important results since all of the species thus obtained were sooner or later taken as adults by net. A representative collection of Odonata was sent to Clarence H. Kennedy and I am indebted to him for many of the determinations used in this paper.

## Suborder ANISOPTERA

## AESCHNIDAE

## AESCHNINAE

- 1 **Anax junius** Drury  
Uncommon. Taken at Orange Grove stream. January 1936.  
Crique Dam. November 1938. Rust-op-twist Pond. March 1941.

## LIBELLULIDAE

## LIBELLULINAE

- 2 *Orthemis ferruginea* F.  
Common along streams and at ponds and watering troughs. 1936.
- 3 *Perithemis domitia* Drury  
Common on streams. 1936.
- 4 *Micrathyrta* sp.  
Adult taken at Jealousy stream. November 1937.
- 5 *Erythrodiplax umbrata* Linn.  
Uncommon. October 1938.
- 6 *Erythrodiplax berenice naeva* Hagen  
Common on coastal marshes. 1935.
- 7 *Erythrodiplax connata justiniana* Selys  
Common on streams. February 1936.
- 8 *Cannacria herbida* Gundlach  
Orange Grove stream. November 1938.
- 9 *Lepthemis vesiculosa* Fabr.  
Common on streams. 1936.
- 10 *Macrothemis celeno* Selys  
Common on streams and ponds. 1936.
- 11 *Dythemis rufinervis* Burm.  
Annaly stream. October 1936.
- 12 *Tamea abdominalis* Ramb.  
Orange Grove stream. November 1938.
- 13 *Pantala flavescens* Fabr.  
Orange Grove stream. December 1938.

## Suborder ZYGOPTERA

## CAENAGRIONIDAE

## CAENAGRIONINAE

- 1 *Telebasis dominicanum* Selys  
Crique Dam. January 1936.
- 2 *Ischnura ramburii credula* Calv.  
Common on ponds. October 1936.
- 3 *Enallagma caecum* Hagen  
Common on streams and ponds. August 1936.

## NEUROPTERA

## CHRYSIDAE

Determinations by Ashley B. Gurney.

1 **Chrysopa** sp.

Abundant on plants *Cryptocordia alba* at night. October 1936.

2 **Myrmeleon insertus** Hagen

Adults taken at light. April 1936.

3 **Vella** sp.

Taken at light. April 1936.

## THYSANOPTERA

## THRIPIDAE

1 **Frankliniella difficilis** Hood

Det. D. Moulton. 1937.

2 **Frankliniella insularis** Franklin

In flowers of *Tabebuia pentaphylla*. 1937. Det. F. Andre.

3 **Heliothrips rubrocinctus** Giard

On avocado tree. April 1937.

## PHLAEOTHIRIPIDAE

1 **Liothrips** sp.

Swarming over rocks on Buck Island. January 1939. Det. F. Andre.

## ANOPLURA

## HAEMATOPINIDAE

1 **Linognathus** sp.

Taken off goats.

2 **Haematopinus** sp.

Common parasite on swine.

3 **Haematopinus tuberculatus** Burmeister

Taken off donkey (*Equus asinus*).

## PEDICULIDAE

1 **Pediculus humanus capitis** DeGeer

Taken from the scalp of man. Det. J. C. Bequaert.

## PHTHIRIDAE

1 **Phthirus pubis** Linn.

Taken off man.

## . HOMOPTERA

## CICADIDAE

1 **Proarna hilaris** Germar

Det. J. S. Caldwell.

## CICADELLIDAE

- 1 **Carneocephala sagittifera** Uhler  
Det. J. S. Caldwell.
- 2 **Sanctanus** sp.  
Det. P. W. Oman.
- 3 **Deltocephalus flavicostus** Stal  
Det. P. W. Oman.
- 4 **Deltocephalus sonorus** Ball  
Det. J. S. Caldwell.
- 5 **Exitianus obscurinervis** Stal  
Det. J. S. Caldwell.
- 6 **Acinopterus angulatus** Lawson  
Det. J. S. Caldwell.
- 7 **Chlorotettix minimus** Baker  
Det. J. S. Caldwell.
- 8 **Chlorotettix tethys** Van D.  
Det. P. W. Oman.
- 9 **Nesosteles bisinuatus** DeL.  
Det. J. S. Caldwell.
- 10 **Nesosteles calcarus** DeL.  
Det. J. S. Caldwell.
- 11 **Nesosteles neglectus** DeL. & D.  
Det. P. W. Oman.
- 12 **Nesostelus incisus** Mats.  
Det. P. W. Oman.
- 13 **Protalebra brasiliensis** Baker  
Det. P. W. Oman.
- 14 **Empoasca** sp.  
Det. J. S. Caldwell.
- 15 **Hortensia similis** Walker  
Det. P. W. Oman.

## FULGORIDAE

- 1 **Catonia** sp.  
Det. P. W. Oman.
- 2 **Bothriocera** sp.  
Det. P. W. Oman.
- 3 **Oliarus complectus** Ball  
Det. J. S. Caldwell.
- 4 **Thionia** sp.  
Det. P. W. Oman.

- 5 **Colpoptera flavifrons** Osborn  
Det. P. W. Oman.
- 6 **Colpoptera maculifrons** Muir  
Det. P. W. Oman.
- 7 **Ormenis marginata** Brunnich  
Det. P. W. Oman.
- 8 **Ormenis quadri-punctata** F.  
Det. J. S. Caldwell.
- 9 **Flatoides** sp.  
Det. P. W. Oman.
- 10 **Perigrinus maidis** Ashmead  
Det. P. W. Oman.
- 11 **Sogata furcifera** Horvath  
Det. J. S. Caldwell.
- 12 **Delphacodes propinqua** Fieber  
Det. J. S. Caldwell.

## APHIDIDAE

- 1 **Aphis rumicis** L.  
Det. P. W. Mason. On *Samanca saman*. 1936.
- 2 **Aphis gossypii** Glover  
On mellon, okra, cotton. 1936.
- 3 **Aphis maidis** Fitch  
Corn. 1939.

## COCCIDAE

The following species were determined by L. O. Howard in 1921.

- 1 **Margarodes formicarum** Guilding  
In the soil in sugar cane fields.
- 2 **Conchaspis angraeci** Cockerell
- 3 **Orthezia praelonga** Douglas
- 4 **Asterolecanium pustulans** Cockerell
- 5 **Pseudococcus nipae** Maskell  
On *Cocos nucifera*, *Psidium guajava*, *Mangifera indica*, *Annona muricata*.
- 6 **Pseudococcus virgatus** Cockerell  
On beans, beets, carrots, cassava, yams, sweet potatoes, tomatoes, cotton, eggplant.
- 7 **Pseudococcus calceolariae** Maskell  
On sugar cane.
- 8 **Pseudococcus citri** Risso  
On citrus, corn.

- 9 **Pseudococcus bromeliae** Bouché
- 10 **Pseudococcus sacchari** Cockerell  
On sugar cane.

## COCCINAE

- 1 **Pulvinaria psidii** Maskell  
On *Psidium guajava*.
- 2 **Pulvinaria urbicola** Cockerell  
On beans, carrots, sweet potatoes.
- 3 **Ceroplastes floridensis** Comstock  
On avocado, *Anacardium occidentale*, *Musa* sp., *Psidium guajava*,  
*Annona muricata*, *Annona squamosa*.
- 4 **Ceroplastes denudatus** Cockerell  
On *Annona squamosa*, *A. muricata*.
- 5 **Vinsonia stellifera** Westwood  
On *Mangifera indica*.
- 6 **Eucalymnus tessellatus** Signoret
- 7 **Coccus hesperidum** L.  
On *Ficus* sp., *Mangifera indica*, *Carica papaya*.
- 8 **Coccus mangiferae** Green  
On *Mangifera indica*.
- 9 **Coccus viridis** Green  
On *Mammea americana*, *Zapota achras*.
- 10 **Saissetia hemisphaerica** Targioni  
On beans, eggplants, okras, *Ficus* sp.
- 11 **Saissetia nigra** Nietner  
On *Persea gratissima*, *Ficus* sp., *Annona muricata*, *Psidium guajava*,  
*Mammea americana*, *Manihot manihot*.
- 12 **Saissetia oleae** Bernard  
On *Anacardium occidentale*, *Persea gratissima*, *Musa* sp., *Psidium guajava*,  
*Ficus* sp. *Mammea americana*, *Annona muricata*, *Zapota achras*.

## DIASPINAE

Determinations by L. O. Howard.

- 1 **Chionaspis citri** Comstock  
On citrus.
- 2 **Howardia biclavis** Comstock  
On *Mammea americana*.
- 3 **Diaspis boisduvalli** Signoret  
On *Musa* sp.

- 4 **Diaspis echinocacti opuntiae** Cockerell  
On cactus.
- 5 **Aulacaspis pentagona** Targioni  
On numerous plants.
- 6 **Hemichionaspis minor** Maskell  
On *Lantana involucrata*.
- 7 **Aspidiotus destructor** Signoret  
On sugar cane, *Cocos nucifera*.
- 8 **Aspidiotus lantaniae** Signoret  
On *Zapota achras*, *Ficus* sp.,
- 9 **Targionia sacchari** Cockerell  
On sugar cane.
- 10 **Targionia hartii** Cockerell  
On *Dioscorea* sp.
- 11 **Lepidosaphes beckii** Newman  
On citrus.
- 12 **Lepidosaphes alba** Cockerell  
On *Manihot manihot*.
- 13 **Ischnaspis longirostris** Signoret  
On *Cocos nucifera*, *Ficus* sp.

## ALEYRODIDAE

Determinations by L. O. Howard.

- 1 **Aleurodicus cocois** Curtis  
On *Cocos nucifera*, *Musa* sp.
- 2 **Bemisia inconspicua** Quaintance  
On cabbage.
- 3 **Aleurothrixus floccosus** Maskell  
On *Psidium guajava*, citrus.

## HEMIPTERA

Harry G. Barber determined the bulk of *Hemiptera* in the collection, J. Bequaert reported on the *Polycetenidae*. Specimens are in U.S.N.M.

## CORIXIDAE

- 1 **Trichocorixa pygmaea** Fieber  
At light. February 1936. In brackish water, marsh, La Grange.  
May 1936.
- 2 **Trichocorixa kollari** Fieber  
In brackish pools, May 1936; Slob Pond, September, 1937.



## BELOSTOMATIDAE

1 **Belostoma boscii** Lepeletier & Serville

Specimens were found hibernating in a dried out mud hole in six inches of moist mud. October 37.

## NOTONECTIDAE

1 **Notonecta indica** L.

Taken in Slob pond. October 1937.

2 **Buenoa femoralis** Fieber

Taken in Rust-op-twist pond. August 1936.

3 **Buenoa pallipes** F.

Taken in Slob pond. October 1937.

4 **Buenoa pallens** Champion

Taken at dusk by net. September 1937. In Slob pond. October 1937.

5 **Buenoa albidus** Champion

By car net at dusk. October 1937.

## VELIIDAE

1 **Microvelia robusta** Uhler

By car net at dusk. September 1937.

## GERRIDAE

1 **Limnogonus franciscanus** Stal

Taken in Slob pond. July 1937.

## MIRIDAE

1 **Reuteroscopus ornatus** Reuter

By car net. October 1937.

2 **Cyrtopeltis varians** Distant

By car net at dusk. October 1937.

3 **Dolichomiris linearis** Reuter

Swept from grass. November 1937.

4 **Rhinacloa forticornis** Reuter

Swept from grass. November 1937.

5 **Sixeonotus** sp.

Swept from grass. November 1937.

## ANTHOCORIDAE

- 1 **Paratriphleps pallidus** Reuter  
By car net at dusk, October 1937.
- 2 **Asthenidea picta** Uhler
- 3 **Asthenidea** sp.
- 4 **Cardiastethus cubanus** Popp  
By car net at dusk. October 1937.
- 6 **Cardiastethus** sp.  
By car net at dusk. October 1937.
- 7 **Lasiochilus divisus** Champion  
By net at dusk. October 1937.
- 8 **Lasiochilus pallidulus** Reuter  
By car net at dusk. October 1937.

## POLYCTENIDAE

- 1 **Hesperoctenes** sp.  
Det. J. Bequaert. Taken off free-tailed bat, *Molossus major*. January 1945.

## CIMICIDAE

- 1 **Cimex hemipterus** F.  
Det. J. Bequaert. Human habitation, October 1935.

## REDUVIIDAE

- 1 **Stenopoda cinerea** Laporte  
Swept from grass. November 1936.
- 2 **Stenopoda culciformis** F.  
Swept from grass. September 1937.
- 3 **Zelus longipes** Linn.  
Swept from shrubbery. October 1936.
- 4 **Empicoris armatus** Champion  
Specimen taken in house. November 1937.
- 5 **Ploiaria bispina** McAtee & Malloch
- 6 **Emesopsis nubilus** Uhler  
At light. December 1936.
- 7 **Triatoma rubrofasciata** DeGreer  
Six specimens taken, caught inside dwellings at night. July 1939, August 1940, September 1942, September 1943, November 1944, March 1945. Microscopic examinations revealed the presence of protozoan parasites of the form *Trypanosoma* in each.

## TINGIDAE

- 1 **Corythucha gossypii** Fabr.  
On cotton.
- 2 **Corythaica planaris** Uhler  
On eggplant. April 1938.
- 3 **Teleonemia sacchari** F.  
Swept by net from grass. July 1936.

## PYRRHOCORIDAE

- 1 **Dysdercus andreae** Linn.  
On cotton. October 1940.

## LYGAEIDAE

- 1 **Oncopeltus fasciatus** Dallas  
Swept from shrubbery. 1937.
- 2 **Oncopeltus aulicus** Fabr.
- 3 **Lygaeus pulchellus** Fabr.
- 4 **Ortholomus jamaicensis** Dallas
- 5 **Ischnorhynchus championi** Distant
- 6 **Blissus leucopterus insularis** Barber
- 7 **Paromius longulus** Dallas
- 8 **Pachybrachius vinctus** Say
- 9 **Pachybrachius bilobatus** Say
- 10 **Ozophora** sp.

## NEIDIDAE

- 1 **Metacanthus decorus** Uhler

## COREIDAE

- 1 **Leptoglossus gonagra** Fabr  
On *Psidium guajava*. October 1935.
- 2 **Leptoglossus stigma** Herbst  
On *Psidium guajava*. October 1936.
- 3 **Phthia picta** Drury  
On watermelons. October 1936.
- 4 **Spartocera fusca** Thunberg  
On *Leucaena glauca*, Mt. Victory. March 1943.
- 5 **Chariesterus gracilicornis** Stal
- 6 **Catorhintha guttula** Fabr.
- 7 **Anasa scorbutica** Fabr.
- 8 **Megalatomus rufipes** Westwood

- 9 *Corizus hyalinus* Fabr.
- 10 *Corizus sidae* Fabr.
- 11 *Jadera rufofusca* Barber

## PENTATOMIDAE

- 1 *Mormidea cubrosa* Dallas
- 2 *Solubea pugnax* Fabr.
- 3 *Euschistus crenator* Fabr.
- 4 *Euschistus* sp.
- 5 *Proxys victor* Fabr.
- 6 *Thyanta casta* Stal
- 7 *Thyanta perditor* Fabr.
- 8 *Loxa* sp.
- 9 *Nezara viridula* L.
- 10 *Acrosternum marginatum* P. B.
- 11 *Piezodorus guildingi* Westwood
- 12 *Arvelius albopunctatus* DeGeer
- 13 *Podisus sagitta* Fabr.

## CYDNIDAE

- 1 *Aethus indentatus* Uhler
- 2 *Amnestus pusio* Stal  
By car net at dusk. November 1937.
- 3 *Amnestus*, probably n. sp.  
By car net at dusk. November 1937.

## SCUTELLERIDAE

- 1 *Tetyra antillarum* Kirk
- 2 *Pachycoris torridus* Scop.  
On wild croton. August 1937.
- 3 *Diolcus boscii* Fabr.
- 4 *Diolcus disjunctus* Barber
- 5 *Diolcus irroratus* Fabr.
- 6 *Augocoris illustris* Fabr.  
On *Sapota achras*, Constitution Hill. June 1935.

## COLEOPTERA

## CICINDELIDAE

- 1 *Tetracha sobrina infusata* Mannerheim  
Det. L. L. Buchanan. Under debris in a coconut grove, Concordia.  
October 1936.

**2 Cicindela boops** Dejean

Det. J. M. Valentine. Taken on the sandy border of a small marsh, Granard. August 1937.

**3 Cicindela trifasciata** Fabr.

Det. J. M. Valentine. Taken on the sandy border of Salt Pond. October 1936.

**4 Cicindela trifasciata tortuosa** Dejean

Det. J. M. Valentine. Taken on the sandy flats of Krause Lagoon. October 1937.

## CARABIDAE

**1 Calosoma granulatum coxale** Mots.

Det. J. M. Valentine. Observed feeding abundantly on the pupae *Laphygma frugiperda* in a field of *Panicum maximum*. August 1938.

**2 Dyschirius** sp.

Det. L. L. Buchanan. 1937.

**3 Clivina insularis** Duval

Det. J. M. Valentine. October 1937.

**4 Clivina** sp.

Det. L. L. Buchanan. By net. October 1937.

**5 Tachys** sp.

Det. L. L. Buchanan. By net. October 1937.

**6 Micratopus insularis** Darlington

Det. J. M. Valentine. By net. 1937.

**7 Micratopus** sp.

Det. L. L. Buchanan. By net. September 1937.

**8 Pseudaptinus** sp.

Det. L. L. Buchanan. By net. October 1937.

**9 Apenes pallipes** Fabr.

Det. L. L. Buchanan. By net. October 1937.

**10 Apenes marginalis** Dejean

Det. L. L. Buchanan. By net. October 1937.

**11 Perigona nigriceps** Dejean

Det. L. L. Buchanan. By net. October 1937.

**12 Pentagonica flavipes** Leconte

Det. L. L. Buchanan. By net. October 1937.

**13 Solenophorus pubifer** Putzeys

Det. L. L. Buchanan. By net. October 1937.

**14 Stelenophorus** sp.

Det. L. L. Buchanan. By net. October 1937.

**15 Stelenophorus** sp.

Det. L. L. Buchanan. By net. October 1937.

## HALIPLIDAE

1 **Haliplus** sp.

Det. L. L. Buchanan. By net. October 1937.

## DYTISCIDAE

Determinations by L. L. Buchanan. Specimens were taken by car net at dusk. October 1937.

- 1 **Rhantus calidus** Fabr.
- 2 **Laccophilus** sp.
- 3 **Canthydrus** sp.
- 4 **Copelatus** sp.
- 5 **Thermonectes** sp.
- 6 **Megadytes**, probably *fraternus* Sharp
- 7 **Celina** sp.
- 8 **Pronoterus** sp.
- 9 **Notomicrus** sp.
- 10 **Bidessus** sp.
- 11 **Eretes sticticus** L.

## GYRINIDAE

1 **Dineutes americanus** L.

Det. L. L. Buchanan. By car net. October 1937.

## HYDROPHILIDAE

Determinations by L. L. Buchanan.

- 1 **Enochrus** sp.  
In pools. October 1937.
- 2 **Berosus** sp.  
In Slob Pond. October 1937.
- 3 **Hydrous insularis** Castelnau  
In Slob pond. October 1937.
- 4 **Hydrous ater** Olivier  
In rain pools, October 1936.
- 5 **Neohydrophilus medius** Brullé  
"Appears to be this species".
- 6 **Tropisternus lateralis** Fabr.  
In rain pools. October 1937.
- 7 **Oosternum costatum** Sharp  
By car net. October 1937.
- 8 **Phaenonotum estriatum** Say  
By car net. October 1937.

9 **Cercyon** sp.

On fungus. January 1939. By car net. October 1937.

10 **Cercyon** sp.

By car net. October 1937.

11 **Cercyon** sp.

By car net. October 1937.

**Paracymnus** sp.

By car net. October 1937.

## STAPHYLINIDAE

All of the Staphylinidae were determined by Richard E. Blackwelder exclusive of a few species which were determined by Edward A. Chapin as indicated. Many of the recorded species were taken by the compiler during the years from 1935 to 1939. An additional number of species were discovered by Blackwelder during a visit to the island from October 23 to December 6, 1936.

Blackwelder, Richard E.: "Monograph of the West Indian Beetles of the Family *Staphylinidae*." United States National Museum Bulletin 182. Washington, D. C., 1943.

*Oxytelinae*1 **Carpelimus fulvipes** Erichson

By car net at dusk. November 1936.

2 **Carpelimus sericeus** Cameron

In bat guano. October.

3 **Carpelimus correctus** Blackwelder4 **Carpelimus beattyi** Blackwelder

In bat guano. November.

5 **Carpelimus imitator** Bierig

Under debris. November.

6 **Carpelimus flavipes** Erichson

By car net at dusk. September.

7 **Carpelimus haplomis** Blackwelder

Flying to light. November.

8 **Apocellus ustulatus** Erichson

In dung. August.

9 **Oxytelus insignitus** Gravenhorst

In dung. October.

10 **Oxytelus incisus** Motschulsky

In dung. November. By car net. October.

11 **Bledius beattyi** Blackwelder

"Collected the type under a stone at the edge of a small stream."

Crique. October.

*Osoriinae*

- 1 **Thoracophorus brevicristatus** Horn  
Under rotting log. November. By car net at dusk. November.
- 2 **Paralispinus exiguus** Erichson  
In fungus. Annaly.

*Steninae*

- 1 **Stenus** sp.  
Det. E. A. Chapin. Under debris, Caledonia. June 1938.

*Paederinae*

- 1 **Lithocharis sororcula** Kraatz  
In dung, flying at dusk. October.
- 2 **Lithocharis secunda** Blackwelder  
In dung. By car net at dusk. November.
- 3 **Lithocharis limbata** Erichson  
In decaying fruit. November.
- 4 **Lithocharis dorsalis** Erichson  
By car net at dusk. November.
- 5 **Stilomedon audanti** Blackwelder  
By car net at dusk. November.
- 6 **Sunius debilicornis** Wollaston  
By car net at dusk. October.
- 7 **Scopaeus** sp.  
Det. E. A. Chapin. In flowers. August, 1938.
- 8 **Lobrathium nitidum** Erichson  
By car net at dusk. November.

*Staphylininae*

- 1 **Philonthus hepaticus** Erichson  
Under beached seaweed. November.
- 2 **Philonthus ventralis** Gravenhorst  
By car net at dusk. November.
- 3 **Philonthus discoidens** Gravenhorst  
In dung. August.
- 4 **Cafius subtilis** Cameron  
Under moist seaweed on beach. November.
- 5 **Cafius caribeanus** Bierig  
Under moist seaweed.
- 6 **Cafius bistriatus** Erichson  
Under moist seaweed.
- 7 **Erichsonius humilis** Erichson  
By car net at dusk. October.



- 8 **Diochus nanus** Erichson  
In rotting fruit. By net at dusk. November.
- 9 **Xantholinus humeralis** Erichson  
In dung. August.
- 10 **Xantholinus beattyi** Blackwelder  
In dung.
- 11 **Leptacinus** sp.  
Det. E. A. Chapin. In debris, Caledonia. June 1938.

*Tachyporinae*

- 1 **Coproporus rutilus** Erichson  
By car net at dusk June 1938.

*Aleocharinae*

- 1 **Gyrophæna** sp.  
Det. E. A. Chapin. In rotting log, Prosperity. April 1938. In fungus, Annaly. November 1938.
- 2 **Atheta** sp.  
Det. E. A. Chapin. In dung. December 1937.

SCAPHIDIIDAE

- 1 **Baeocera** sp.  
Det. H. S. Barber.

HISTERIDAE

- 1 **Hister confinis** Er  
Det. H. S. Barber. In debris. December, 1937.
- 2 **Hister** sp.  
Det. H. S. Barber.

LYCIDAE

- 1 **Thonalmus** sp.  
Det. H. S. Barber. On shrubbery. August 1936.
- 2 **Leptolycus** sp.  
Det. H. S. Barber. On shrubbery. April 1935.

LAMPYRIDAE

- 1 **Pyractomena galeata** E. Olivier  
Det. H. S. Barber. East End. July 1935.

CANTHARIDAE

- 1 **Belotus** sp.  
Det. H. S. Barber. By car net at dusk. October 1937.

## OEDEMERIDAE

1 **Oxacis** sp.

Det. H. S. Barber. By car net at dusk. October 1937.

2 **Ananca vittata** Fabricius

Colony in house, Cane Bay. April 1936.

## MELOIDAE

1 **Zonitis annulicornis** Chevrolat

Det. H. S. Barber. By net at dusk. October.

## ELATERIDAE

1 **Chalcolepidius virginalis** Candèze

Det. J. M. Valentine. At night. October.

2 **Aeolus** sp.

Det. W. S. Fisher. On mangrove bush. October 1937.

3 **Conoderus bifoveatus** Beauvois

Det. W. S. Fisher. Under scaling bark. October.

4 **Conoderus lividus** DeGeer

Det. J. M. Valentine.

## BUPRESTIDAE

1 **Polycesta thomae** Chevrolat

Det. W. S. Fisher.

## DERMESTIDAE

1 **Globicornis fulvipes** Guérin

Det. H. S. Barber. By car net at dusk. November 1937.

2 **Attagenus** sp.

Det. H. S. Barber. By car net at dusk. October 1939.

## NITIDULIDAE

Determinations by Edward A. Chapin.

1 **Carpophilus** sp.

By car net at dusk. November 1938.

2 **Haptoncus luteolus** Erichson

In ripe fruit of *Anacardium occidentale*. July.

3 **Stelidota geminata** Say

On fruit of *Spondias purpurca*. May 1935.

4 **Stelidota** sp.

By car net at dusk. October 1937.

5 **Lobiopa insularis** Castelnar

In ripe fruit of Mango, *Spondias mombin*, *Spondias purpurea*. September 1941.

6 **Epuraea** sp.

## MONOTOMIDAE

1 **Europs** sp.

Det. W. S. Fisher. By car net at dusk. October 1937.

## CUCUJIDAE

1 **Monanus concinnulus** Walker

Det. W. S. Fisher.

2 **Telephanus pallidulus** Chevrolat

Det. W. S. Fisher. By car net. October.

## CRYPTOPHAGIDAE

Determinations by W. S. Fisher.

1 **Cryptophagidae** Gen & sp.?2 **Loberus testaceus** Reitter

By car net at dusk. September 1937.

3 **Loberus** sp.

By car net at dusk. September 1937.

4 **Ephistemus** sp.

By car net at dusk. September 1937.

5 **Hapalips** sp.

By car net at dusk. September 1937.

## MYCETOPHAGIDAE

1 **Typhaea stercorea** L.

Det. W. S. Fisher. By car net at dusk. November 1937.

## PHALACRIDAE

1 **Phalacrus** sp.

Det. W. S. Fisher. By car net at dusk. September 1939.

## COCCINELLIDAE

Determinations by Edward A. Chapin.

1 **Scymnus** sp.

On shrubbery. October 1937.

2 **Decadiomus** sp.

On shrubbery. November 1939.

**3 Scymnillodes sp.**

By car net at dusk. October 1939.

**4 Psyllobora lineola F.**

By car net at dusk. November 1939.

**5 Coleomegilla cubensis Casey**

By car net at dusk. October 1939.

**6 Cycloneda sanguinea limbifer Casey**

By car net. October 1937.

**7 Azya sp.****8 Naemia seriata Melsheimer**

## ALLECULIDAE

**1 Hymenorus sp.**

Det. E. A. Chapin.

## TENEBRIONIDAE

**1 Opatrinus anthracinus Mulsant**

Det. R. E. Blackwelder.

**2 Opatrinus gemellatus Olivier**

Det. E. A. Chapin. By net. October 1939.

**3 Blapstinus n.sp.**

Det. E. A. Chapin. By net. October 1937.

**4 Blapstinus n. sp.**

Det. E. A. Chapin. By net. October 1939.

**5 Phaleria variabilis Quedenfeldt**

Det. E. A. Chapin. By net. October 1939.

**6 Diaperis maculata Olivier**

Det. R. E. Blackwelder.

**7 Tribolium castaneum Herbst**

Det. E. A. Chapin. By net. October 1939.

**8 Alphetobius diaperinus Panzer**

Det. R. E. Blackwelder.

**9 Alphetobius piceus Olivier**

Det. R. E. Blackwelder.

**10 Alphetobius sp.**

Det. R. E. Blackwelder.

**11 Eutomus sp.**

Det. E. A. Chapin.

**12 Zophobas morio F.**

Det. E. A. Chapin.

**13 Prateus sp.**

Det. E. A. Chapin. By car net at dusk. October 1939.

14 **Trachyscelis** sp.

Det. E. A. Chapin. By car net at dusk. October 1939.

## PTINIDAE

1 **Gibbium psylloides** Czemp.

Det. W. S. Fisher.

2 **Ptinus** sp.

Det. W. S. Fisher. By car net at dusk. October 1937.

## ANOBIIDAE

1 **Anobiidae** Gen & sp.

Det. W. S. Fisher. By car net at dusk. October 1937.

## BOSTRYCHIDAE

Determinations by W. S. Fisher.

1 **Apate monachus** F.

By car net at dusk. September 1937.

2 **Tetrapriocera tridens** F.

Under log. September 1937.

3 **Xylomeira torquata** F.

## CISIDAE

1 **Cis hirtellus** Jacq. Duval

Det. J. M. Valentine.

2 **Cis** sp.

Det. W. S. Fisher. By car net at dusk. October. On fungus.  
January 1939.

3 **Ceracis** sp.

Det. W. S. Fisher. On fungus. November 1938.

## SCARABAEIDAE

Determinations by E. A. Chapin.

1 **Aphodius lividus** Olivier

In dung. December 1937.

2 **Aphodius cuniculus** Chevrolat

In dung. April 1941.

3 **Pleurophorus parvulus** Chevrolat4 **Ataenius frater** Leconte

In dung. April 1941.

5 **Ataenius edwardsi** Chapin

In dung. June 1939.

- 6 **Ataenius gracilis** Melsheimer  
By car net at dusk. October 1939.
- 7 **Ataenius miamii** Cartw.  
By car net at dusk. October 1939.
- 8 **Ataenius darlingtoni** Hntn.  
By car net at dusk. October 1939. In manure. December 1937.
- 9 **Ataenius imbricatus** Melsheimer  
In dung. April 1941.
- 10 **Ataenius strigicauda** Bates  
By car net at dusk. September 1937.
- 11 **Ataenius beattyi** Chapin  
In dung. April 1938.
- 12 **Trox suberosus** F.  
Under debris. August 1936. At light. November 1944.
- 13 **Phyllophaga microphylla** Moser  
Feeding at night on leaves of *Terminalia catappa*. September 1938.  
On leaves of *Genipa americana*. August.
- 14 **Ligyris tumulosus** Burmeister  
At light. September 1936.
- 15 **Ligyris cuniculus** F.  
At light. September 1936.
16. **Strataegus barbigerus** Chapin  
At light. October 1936.

## CERAMBYCIDAE

Determinations by W. S. Fisher.

- 1 **Methia necydalea** F.
- 2 **Chlorida festiva** L.
- 3 **Eburia decemmaculata** F.  
At light. September 1936.
- 4 **Elaphidion insulare** Neuman  
Larvae in branch of *Annona squamosa*. August 1935.
- 5 **Heterachthes cylindricollis** F.
- 6 **Heterachthes quadrimaculatus** F.  
At light. September 1936.
- 7 **Stizocera vanzwaluwenburgi** Fisher
- 8 **Cylindera flava** F.  
At light. September 1936.
- 9 **Lagochirus araneiformis** Linn.
- 10 **Leptostylus testaceus** Froelich  
At light. September 1936.

- 11 **Lepturges guadeloupensis** Fleutiaux & Sallé
- 12 **Ataxia alboscuteolata** Fisher  
By net. September 1936.
- 13 **Estola** sp.  
"Probably a new species but do not like to describe it on a single specimen."

## CHRYSOMELIDAE

Determinations by H. S. Barber.

- 1 **Lema** sp.
- 2 **Nodonota** sp.  
By car net at dusk. November 1937.
- 3 **Cerotoma ruficornis** Olivier  
By car net at dusk. October 1937.
- 4 **Homophoeta albicollis** F.  
By car net at dusk. October 1939.
- 5 **Oedionychis bicolor** L.
- 6 **Podagric**a sp.
- 7 **Altica**, near *purpurescens* Suffrian  
On shrubbery. September 1938.
- 8 **Altica** sp.  
On shrubbery. September 1938.
- 9 **Chalepus sanguinicollis** L.  
By car net at dusk. November 1937.
- 10 **Phyllotreta fallax** Suffrian
- 11 **Deloyala guttata** Olivier  
Swept from shrubbery. October 1937.

## BRUCHIDAE

- 1 **Callosobruchus maculatus** F.  
Det. H. S. Barber. November 1939.

## BRENTIDAE

- 1 **Brentus volvulus** L.  
Under scaling bark of decaying tree. November 1939.

## CURCULIONIDAE

Determinations by L. L. Buchanan.

- 1 **Artipus** sp.  
September 1937.
- 2 **Diaprepes abbreviatus rohrii** F.  
On shrubbery. September 1937.

- 3 **Lachnopus curvipes** F.  
On acacia bush. November 1938.
- 4 **Apodrusus**, near **argentatus** Wolcott
- 5 **Anthonomus** sp.  
Reared from larvae in ripe fruit of *Malpighia punicifolia*. May 1941.
- 6 **Anacentrinus** sp.  
By net. September 1937.
- 7 **Sternechus** sp.
- 8 **Chalcodermus** sp.  
Eaten by frog *Eleutherodactylus lentus*. August 1936.
- 9 **Chalcodermus ebeninus** Boheman  
On corn. July 1938.
- 10 **Euscepes batata** Waterhouse  
In sweet potatoes. February 1936.
- 11 **Anchonus** sp.  
On shrubbery. September 1937.
- 12 **Cassonus canaliculatus** F.  
On shrubbery. September 1937.
- 13 **Metamasius hemipterus** Linn.  
On shrubbery. September 1938. Specimens were infested with *Analgasid* mites attached to thorax.
- 14 **Cosmopolites sordidus** Germar  
January 1936. From roots of banana.
- 15 **Calendra** (**Calandra**) **oryzae** Linn.  
In stored corn.
- 16 **Cryptorhynchinae**  
"Genus not found."

## SCOLYTIDAE

Determinations by M. W. Blackman.

- 1 **Hypothenemus flavipes** Hopkins  
In seed pod of *Albizia lebbek*. April 1936.
- 2 **Coccotrypes** sp.  
By car net at dusk. October 1937.
- 3 **Xyleborus affinis** Eichoff  
By car net at dusk. October 1937.
- 4 **Xyleborus confusus** Eichoff  
By car net at dusk. October 1937.
- 5 **Xyleborus sacchari** Hopkins  
Car net at dusk. October 1937.
- 6 **Xyleborus propinquus** Eichoff  
Car net at dusk. October 1937.



## DIPTERA

I am greatly indebted to Alan Stone for his determinations in the *Culicidae* at a time when I was engaged in Malaria control and Filaria studies with the Department of Health. The *Culicoides* were examined by the late W. A. Hoffman. The bulk of Diptera was determined by C. T. Greene and D. G. Hall. A small collection of *Hippoboscidae* and *Streblidae* was determined by J. Bequaert.

## TIPULIDAE

Determinations by Alan Stone.

- 1 **Limonia (Rhipidia) domestica** Osten Sacken  
December 1937.
- 2 **Limonia (Geranomyia) antillarum** Alexander  
At light. December 1937.
- 3 **Limonia (Geranomyia) virescens** Loew  
December 1937.
- 4 **Limonia** sp.  
Decembr 1937.
- 5 **Limonia** sp.  
December 1937.

## PSYCHODIDAE

- 1 **Psychodidae** Gen & sp.  
Det. C. T. Greene.

## CHIRONOMIDAE

- 1 **Chironomidae** Gen & sp.  
Det. C. T. Greene. October 1935.
- 2 **Chironomus** sp.  
Det. A. Stone. October 1935.
- 3 **Chironomus redeuns** Walker  
Det. C. T. Greene. August 1935.

## CERATOPOGONIDAE

- 1 **Culicoides furens** Poey  
Det. W. A. Hoffman. A vicious feeder. July 1935.
- 2 **Culicoides laughnani** Edwards  
Det. W. A. Hoffman. Found breeding in fresh water puddles near streams. May 1936, August 1936.
- 3 **Culicoides** sp. near **laughnani**  
Det. W. A. Hoffman. August 1938.

4 **Culicoides** n. sp.

Det. W. A. Hoffman. Taken at Crique Dam. January 1936.  
Constitution Hill. May 1936, January 1937. Diamond. September 1938.

5 **Forcipomyia propinqua** Williston

Swarming at dusk. October 1937. Det. Alan Stone.

6 **Forcipomyia raleighi** Macfie

Det. H. K. Townes. October 1936.

7 **Forcipomyia** sp.

Det. A. Stone. December 1937.

8 **Dasyhelea** sp.

Det. A. Stone. Peters Rest School. December 1937.

9 **Bezzia setulosa** Loew

Peters Rest School, December 1937. Det. A. Stone.

## CULICIDAE

Determinations by Alan Stone.

1 **Corethrella appendiculata** Grabham

Reared from larvae in tree holes. October 1935.

2 **Anopheles albimanus** Wiedemann

Larvae found wherever there are collections of fresh water, preferably in unshaded situations. October 1935.

3 **Anopheles grabhamii** Theobald

Strictly a fresh water species, the larvae found in shaded pools and sluggish streams. October 1935.

4 **Uranotaenia loewii** Theobald

Uncommon. Larvae collected in fresh water pools. September 1935.

5 **Uranotaenia cooki** Root

Rare, larvae found in shaded pools. October 1936.

6 **Psorophora jamaicensis** Theobald

Larvae in fresh rain pools in pasturelands. August 1935.

7 **Psorophora pygmaea** Theobald

Rain pools and near the coast in slightly brackish pools. October 1935.

8 **Psorophora insularia** Dyar & Knab

Adults were taken by net at Williams Pond. November 1935.

9 **Psorophora coffini** Dyar & Knab

Adults by net. Christiansted. October 1935.

10 **Aedes taeniorhynchus** Wiedemann

Larvae in fresh water pools. Adults biting by day. September 1935.

- 11 **Aedes mediovittatus** Coquillett  
Larvae in water holes in trees, adults biting by day. Common in jungles. October 1935.
- 12 **Aedes aegypti** L  
Common about dwellings. Breed in fresh water. 1935.
- 13 **Aedes scapularis** Rondani  
Larvae in rain pools. September 1936.
- 14 **Aedes sollicitans** Walker  
Larvae in slightly brackish rain pools at Liaka. November 1936.
- 15 **Aedes tortilis** Theobald  
Larvae in rain pools. East End. August 1935.
- 16 **Culex bahamensis** Dyar & Knab  
Larvae in black masses in brackish pools near the coast. August 1935.
- 17 **Culex habilitator** Dyar & Knab  
Common. Breeds in fresh water. September 1935.
- 18 **Culex nigripalpus** Theobald  
Breeds in fresh water and sometimes the larvae are found in slightly brackish pools. May 1935.
- 19 **Culex quinquefasciatus** Say  
Breeds in fresh water, rarely in slightly brackish pools. September 1935.
- 20 **Culex atratus** Theobald  
Larvae found in fresh and slightly brackish pools. March 1935.
- 21 **Culex erraticus** Dyar & Knab  
Larvae in fresh water pool. December 1935.
- 22 **Culex infictus** Theobald  
In fresh water pools. January 1937.
- 23 **Culex americanus** N. L.  
Adults were taken from a rotting palm trunk which had become hollowed out. Crique. 1938.
- 24 **Culex chrysonotum** Dyar & Knab  
Larvae from fresh water pool. September 1935.
- 25 **Culex** sp.  
Captured on arm, Fairplain. February 1936. Crique. August 1938.
- 26 **Deinocerites cancer** Theobald  
Breeds in slightly brackish water, preferably down in large land-crab holes. November 1935.

#### MYCETOPHILIDAE

- 1 **Mycetophila insipiens** Williston  
Det. A. Stone. 1935.

2 **Sciara** sp.

Det. A. Stone.

## CECIDOMYIDAE

1 **Diplosis** sp.

Det. C. T. Greene. From sorghum. April 1936.

2 **Cecidomyidae** gen. & sp.

Det. C. T. Greene. 1936.

## SIMULIIDAE

1 **Simulium quadrivittatum** LoewAdults taken on arm biting man. Not a common species. Crique.  
1936.

## STRATIOMYIDAE

Determinations by C. T. Greene.

1 **Hermetia illucens** Linn.Preyed upon by *Stictia signata*. January 1936.2 **Neorondania chalybea** Wiedemann 19363 **Cyphomyia marginata** Loew

Resting on orange leaves. May 1937.

4 **Cyphomyia lasiophthalmus** Williston

September 1938.

5 **Euryneurasoma slossonae** Johnson

August 1937.

## TABANIDAE

1 **Stenotabanus stigma** F.

Det. A. Stone. Taken on dog. August 1941.

## BOMBYLIIDAE

Determinations by C. T. Greene.

1 **Anthrax aedipus** Fabr.

August 1936.

2 **Anthrax** sp.

August 1936.

3 **Hyperalonia cerberus** Fabr.

August 1936.

4 **Villa fauna** Fabr.

May 1938.

5 **Bombylius** sp.

## ASILIDAE

- 1 **Leptogaster** sp.  
April 1938.
- 2 **Ommatius marginellus** Fabr.  
April 1936.

## DOLICHOPODIDAE

Determinations by C. T. Greene.

- 1 **Chrysotus** sp.  
April 1936.
- 2 **Gymnopternus** sp.  
"Shining green." May 1936, August 1936, June 1941.
- 3 **Plagioneurus univittatus** Loew  
On swamp grass. May 1936.
- 4 **Pelastoneurus unguiculatus** Aldrich  
On swamp grass. May 1936.
- 5 **Dolichopodidae** gen & sp.  
"Near Mesorhaga." On grass. May 1936.
- 6 **Sciapus graenicheri** Van Duzee  
September 1940.

## EMPIDIDAE

- 1 **Drapetis** sp.  
Det. C. T. Greene.

## PHORIDAE

- 1 **Megaselida scalaris** Loew  
February 1936. Det. C. T. Greene.
- 2 **Megaselida** sp.  
Det. C. T. Greene. Reared from larvae in rotting fruit of  
*Anacardium occidentale*. June 1937.
- 3 **Puliciphora** sp.  
September 1936.

## SYRPHIDAE

Determinations by C. T. Greene. Specimens swept from grass.

- 1 **Baccha clavata** F.  
March 1937.
- 2 **Baccha conformis** Loew  
November 1936.
- 3 **Baccha cylindrica** F.  
August 1936.

- 4 **Baccha dimidiata** F.  
August 1936.
- 5 **Baccha latiusculus** Loew  
April 1936.
- 6 **Baccha stenogaster** Williston  
August 1936.
- 7 **Toxomerus geminatus** Say  
September 1941.
- 8 **Toxomerus politus** Say  
August 1938.
- 9 **Toxomerus** sp.  
"Banded abdomen." Grove Place. January 1941.
- 10 **Allograpta fuscisquama** Curran  
September 1936.
- 11 **Allograpta venusta** Curran  
August 1936.
- 12 **Volucella unipunctata** Curran  
September 1937.
- 13 **Eristalis vinetorum** Fabr.  
October 1940.

## TACHINIDAE

Determinations by D. G. Hall.

- 1 **Tachinidae** gen & sp.  
August 1936.
- 2 **Trichopoda haiiensis** Desvoidy  
October 1936
- 3 **Trichopoda** sp.  
Preyed upon by *Stictia signata*. November 1936.
- 4 **Lydella bigeminata** Curran  
August 1936.
- 5 **Ocyptera** sp.  
August 1936.
- 6 **Nemorilla maculosa** Macquart  
October 1938.
- 7 **Phorocera divisa** Aldrich & Webber  
Adults in dwellings. May 1941.
- 8 **Argyrophylax** sp.  
April 1936, August 1936.
- 9 **Ormia** sp.  
April 1936.

- 10 **Gonia** sp.  
May 1941.

## SARCOPHAGIDAE

- 1 **Sarcophagula occidua** Fabr.  
January 1936. Det. D. G. Hall.  
2 **Sarcophaga hillifera** Aldrich  
Det. M. T. James. October 1943.  
3 **Sarcophaga** sp.  
Det. D. G. Hall. November 1936.  
4 **Helicobia surrubea** V. de W.  
March 1936.

## MUSCIDAE

- 1 **Muscidae**  
Genus uncertain. Det D. G. Hall. October 1938.  
2 **Cochliomyia macellaria** F.  
Reared from larvae taken off dead animals. Det D. G. Hall.  
3 **Lucilia eximia** Macquart  
Det. M. T. James. August 1936.  
4 **Lucilia** sp.  
Det. D. G. Hall. Preyed upon by *Stictia signata*. November 1936.  
5 **Pyrellia** sp.  
Det. D. G. Hall. August 1936.  
6 **Morellia scapulata** Bigot  
Det. D. G. Hall. March 1936.  
7 **Orthellia** sp.  
Det. D. G. Hall. September 1937.  
8 **Synthesiomyia nudiseta** V de W.  
Det. M. T. James. October 1938.  
9 **Musca domestica** L.  
Det. D. G. Hall. November 1936.  
10 **Haematobia irritans** L.  
Annoying domestic animals. Det. D. G. Hall. 1941.  
11 **Muscinae** gen & sp.  
Det. D. G. Hall. On fruit of *Calyptrocordia alba*. 1936.

## ANTHOMYIIDAE

Determinations by D. G. Hall.

- 1 **Anthomyiidae** gen & sp.  
Swept from grass. August 1936.

- 2 **Anthomyiidae** gen & sp.  
August 1936.
- 3 **Anthomyiidae** gen & sp.  
Hovering about dead horse. April 1936.
- 4 **Anthomyia** sp.  
Swarming. June 1941.
- 5 **Atherigona orientalis** Schiner  
September 1938.
- 6 **Lispa** sp.  
August 1936.
- 7 **Phaonia** sp.  
April 1936.
- 8 **Fannia femoralis** Stein  
November 1936.

## BORBORIDAE

- 1 **Leptocera** sp.  
Det. D. G. Hall. July 1938.
- 2 **Leptocera discalis** Malloch  
October 1937.

## SAPROMYZIDAE

Determinations by D. G. Hall. Material swept from vegetation.

- 1 **Sapromyzidae**  
Probably *Camptoprosopella* sp. August 1936.
- 2 **Carpolonchaea pendula** Bezzi  
June 1941.
- 3 **Neogriphoneura sordida** Wiedemann  
September 1938.
- 4 **Sapromyza** sp.  
June 1941.
- 5 **Minettia** sp.  
April 1941.
- 6 **Minettia slossonae** Coquillett  
April 1941.

## ORTALIDAE

Determinations by C. T. Greene. Material swept from vegetation.

- 1 **Acrosticta** n. sp.  
"Red abdomen." June 1941.
- 2 **Euxesta annonae** Fabr.  
June 1941.



- 3 **Euxesta spoliata** Loew  
June 1941.
- 4 **Euxesta thomae** Loew  
June 1941.
- 5 **Euxesta stigmatias** Loew  
June 1941.
- 6 **Euxesta sororcula** Wiedemann  
June 1941.

## TRYPETIDAE

- 1 **Anastrepha mombinpraeoptans** Sein  
Det. A. Stone. Reared from larvae in *Spondias mombin*, August-October, 1940. Reared from *Spondias purpurea*.
- 2 **Ensina piccicola** Bigot  
Det. C. T. Greene. September 1942.
- 3 **Trypanea dacetoptera** Phillips  
Det. C. T. Greene. June 1943.

## SEPSIDAE

- 1 **Sepsis pusio** Schiner  
Det. D. G. Hall. August 1936.
- 2 **Sepsis** sp.  
Det. D. G. Hall. August 1936.

## EPHYDRIDAE

Determinations by D. G. Hall. Material swept from grass.

- 1 **Ephydridae** gen & sp.  
May 1937.
- 2 **Discomyza dubia** Williston  
November 1939.
- 3 **Discomyza maculipennis** Wiedemann  
November 1939.
- 4 **Paralimna obscura** Williston  
September 1938.
- 5 **Psilopa nigrimana** Williston  
May 1937.
- 6 **Psilopa** sp.  
June 1941.
- 7 **Ceropsilopa** sp.  
December 1940.
- 8 **Plagiops nitidifrons** Cresson  
June 1940.

9 **Scatella** sp.

November 1946.

## CHLOROPIDAE

1 **Chloropidae** gen & sp.

Det. D. G. Hall. October 1939.

2 **Hippelates apicata** Malloch

On sores. Det. C. W. Sabrosky. October 1939.

3 **Hippelates peruanus** Becker

On dogs. Det. C. W. Sabrosky. November 1939.

4 **Hippelates lutzi** Curran

Det. C. W. Sabrosky. On sores. October 1939.

5 **Hippelates currani** Aldrich

Det. C. W. Sabrosky. October 1939.

6 **Hippelates** sp. near **impressus** Becker

On sores. Det. D. G. Hall. October 1939.

7 **Pseudogaurax lancifer** Coquillett

Det. D. G. Hall. Reared from egg sacs of spider. August 1941.

8 **Conioscinella mars** Curran

Det. C. W. Sabrosky. October 1939.

9 **Oscinellinae** gen & sp.

Det. D. G. Hall. April 1936.

10 **Oscinella forbesi** Curran

Det. C. W. Sabrosky. April 1936.

11 **Oscinella sicatrix** Curran

Det. C. W. Sabrosky. April 1936.

12 **Oscinella** sp

Det. D. G. Hall. April 1936.

## ASTEIIDAE

1 **Sigaloessa**

"Possibly this genus." Det. C. W. Sabrosky. November 1939.

## DROSOPHILIDAE

Determinations by C. T. Greene. Material swept from shrubbery.

1 **Drosophila melanogaster** Meigen

June 1941.

3 **Drosophila repleta** Wollaston

July 1935.

4 **Drosophila busckii** Coquillett

August 1936.

- 5 **Drosophila similis** Williston  
September 1941.
- 6 **Drosophila** sp.  
April 1936.
- 7 **Drosophila** sp.  
March 1941.
- 8 **Cladochaeta nebulosa** Coquillett  
July 1939.
- 9 **Leucophenga** sp. near **varia** Walker  
September 1936.

## AGROMYZIDAE

- 1 **Agromyza viridula** Coquillett  
Det. C. T. Greene.
- 2 **Agromyza virens** Loew  
Det. C. T. Greene.
- 3 **Agromyza longicauda** Curran  
Det. C. T. Greene.
- 4 **Agromyza** sp.  
Det. C. T. Greene. June 1941.

## MILICHIIDAE

- 1 **Milichiella lacteipennis** Loew  
Det. D. G. Hall. March 1939.
- 2 **Milichiella** sp.  
Det. C. T. Greene. August 1939.
- 3 **Desmometopa M-nigrum** Zetterstedt  
Det. C. T. Greene. March 1939.

## HIPPOBOSCIDAE

- 1 **Ornithoetona erythrocephala** Leach  
Taken off Red-tailed Hawk, *Buteo j. jamaicensis*. Det. J. Bequaert.  
1937.
- 2 **Pseudolynchia maura** Bigot  
Off Ground Dove, *Columbigallina passerina nigrirostris*. Det. A.  
Stone. 1936.
- 3 **Microlynchia pusilla** Speiser  
Off Ground Dove, *Columbigallina passerina nigrirostris*. Det. A.  
Stone. 1936.
- 4 **Olfersia aenescens** Thompson  
Off White-bellied Booby, *Sula leucogaster*. Det. J. Bequaert. 1936.

- 5 **Olfersia fossulata** Macquart  
Taken by net from bush. Det. J. Bequaert. 1936.
- 6 **Olfersia spinifera** Leach  
Off Frigate-bird, *Fregata magnificens rothschildi*. Det. J. Bequaert. 1936.
- 7 **Olfersia erythropsis** Bigot  
Off Blue-faced Booby, *Sula d. dactylatra*. Det. A. Stone. 1936.
- 8 **Olfersia sordida** Bigot  
By net on shrubbery. Det. A. Stone. April 1936.
- 9 **Melophagus ovinus** Linn.  
Off domestic sheep. 1936.

## STREBLIDAE

Determinations by J. Bequaert.

- 1 **Trichobius truncatus** Kessel  
Off fruit bat, *Brachyphylla cavernarum*. 1938.
- 2 **Trichobius mixtus** Curran  
Off fruit bat, *Artibeus jamaicensis*. 1945.
- 3 **Trichobius sparsus** Kessel  
Off fruit bat, *Artibeus jamaicensis*. 1945.
- 4 **Pterellipsis aranea** Coquillett  
Off fruit bat, *Artibeus jamaicensis*. 1945.

## SIPHONAPTERA

## TUNGIDAE

- 1 **Tunga penetrans** Linn.  
Taken from man.
- 2 **Echidnophaga gallinacea** Westwood  
Off rat (*Rattus fatus alexandrinus*).

## PULICIDAE

- 1 **Pulex irritans** Linn.  
Taken off man.
- 2 **Xenopsylla cheopis** Rothschild  
Off gray rat (*Rattus r. alexandrinus*).
- 3 **Ctenocephalides canis** Curtis  
Common on dogs, mongoose (*Herpestes birmanicus*), and occasionally on man.
- 4 **Ctenocephalides felis** Bouché  
Taken off cats, dogs and mongoose (*Herpestes birmanicus*).

## LEPIDOPTERA

The compiler made determinations in the Rhopalocera after Comstock, and a few species also in *Arctiidae*, *Sphingidae* and *Noctuidae*. However a collection of the microlepidoptera was examined by W. T. Forbes and he has identified the Lesser Antillean form of the monarch butterfly. The bulk of the material was worked over by W. D. Field, C. Heinrich, J. F. Gates Clarke and H. W. Capps and I am indebted to them all for their labors.

## Suborder RHOPALOCERA

## DANAIDAE

- 1 **Danaus plexippus leucogyne** Butler  
November 1936.
- 2 **Danaus plexippus megalippe** Hubner  
Det. W. T. Forbes. November 1944.

## NYMPHALIDAE

- 1 **Heliconius c. charithonius** L.  
February 1936.
- 2 **Heliconius charithonius punctatus** Hall  
February 1936.
- 3 **Dione vanillae insularis** Maynard  
November 1936.
- 4 **Junonia evarete zonalis** C. R. Felder  
This is the common form. October 1936.
- 5 **Junonia evarete incarnata** C. R. Felder  
This species is restricted to the coastal area where it is not uncommon in the salt marshes, but is extremely active and difficult to capture.  
November 1936.
- 6 **Anartia jatrophae intermedia** Munroe  
Common. November 1936.
- 7 **Metamorpha stelenes stelenes** L.  
Common in woods. October 1936.
- 8 **Biblis hyperia hyperia** Cramer  
Common in the woods. October 1936.
- 9 **Hypolimnys missippus** L.  
Rare. Only six specimens have been taken and all of these were in the fall of the year. October 1936.
- 10 **Eunica tatila tatilista** Kaye  
A rare fly. August 1937.

**11 *Anaea troglodyta astina* Fabr.**

A rare butterfly which has been taken only four times and in the fall of the year. August 1935.

## LYCAENIDAE

**1 *Thecla acis mars* Fabr.**

December 1936.

**2 *Thecla angelia boyeri* Comstock & Huntington**

November 1936.

**3 *Thecla simaethis simaethis* Drury**

January 1936.

**4 *Thecla bubastus ponce* Comstock & Huntington**

October 1936.

**5 *Thecla antiqua* Comstock & Huntington**

November 1936.

**6 *Leptotes cassius catilina* Fabr.**

November 1936.

**7 *Hemiargus ammon woodruffi* Comstock & Huntington**

September 1936.

**8 *Hemiargus hanno watsoni* Comstock & Huntington**

September 1936.

## PIERIDAE

**1 *Phoebis (Phoebis) sennae sennae* L.**

September 1936.

**2 *Eurema (Eurema) दौरा ebriola* Poey**

October 1937.

**3 *Eurema (Eurema) elathea* Cramer**

September 1938.

**4 *Eurema (Pyrisitia) lisa euterpe* Ménétries**

February 1936.

**5 *Appias (Glutophrissa) drusilla boydi* Comstock**

A rare species. January 1936.

**6 *Ascia monuste eubotea* Latreille**

Common. October 1936.

**7 *Ascia monuste virginia* Latreille**

Common. September 1936.

## PAPILIONIDAE

**1 *Papilio polydamus thymus* Rothschild & Jordan**

January 1936.

## HESPERIIDAE

- 1 **Polygonus lividus** Hübner  
August 1936.
- 2 **Urbanus proteus** L.  
A common species, larvae parasitized by *Apanteles leucostigma*.  
November 1936.
- 3 **Urbanus proteus cramptoni** Comstock  
This species has not been collected here.
- 4 **Pyrgus syrichtus** Fabr.  
Common. August 1935.
- 5 **Achlyodes papinianus minor** Comstock  
January 1936.
- 6 **Ephyriades arcas** Drury  
October 1936.
- 7 **Wallengrenia otho mutchleri** Watson  
November 1937.
- 8 **Choranthus vitellius** Fabr.  
September 1936.
- 9 **Lerodea tripuncta** Herrich-Schaffer  
October 1936
- 10 **Calpodes ethlius** Cramer  
November 1937.
- 11 **Panoquina nyctelia** Latreille  
October 1936.
- 12 **Panoquina sylvicola woodruffi** Watson  
December 1937.
- 13 **Panoquina p. panoquinoides** Skinner  
September 1938.

## Suborder HETEROCERA

## AMATIDAE

- 1 **Mollodeta partheni** F.  
Det. W. D. Field. February 1937.
- 2 **Cosmosoma achemon tyrrhene** Hübner  
Det. W. D. Field. November 1937.
- 3 **Horama** sp.  
Det. W. D. Field. February 1937.

## ARCTIIDAE

- 1 **Ecpantheria icasia** Cramer  
February 1936.

**2 *Utetheisa ornatix ornatix* L.**

November 1935.

## NOCTUIDAE

**1 *Heliothis obsoleta* F.**

Det. L. O. Howard. Experiment Station collection.

**2 *Heliothis virescens* F.**

Det. C. Heinrich. December 1936.

**3 *Xanthopastis timais* Cramer**

December 1936.

**4 *Leucania humidicola* Guenée**

Det. C. Heinrich. December 1936.

**5 *Leucania multilinea* Walker**

Det. C. Heinrich. December 1936.

**6 *Perigea cupentia* Cramer**

Det. C. Heinrich. December 1936.

**7 *Perigea concisa* Walker**

Det. C. Heinrich. December 1936.

**8 *Catabena vitrina* Walker**

Det. C. Heinrich. December 1936.

**9 *Prodenia ornithogalli* Guenée**

Det. L. O. Howard. Exp. Station coll.

**10 *Laphygma frugiperda* Smith & Abbot**

Det. C. Heinrich. December 1936.

**11 *Xylomiges sunia* Guenée**

Det. L. O. Howard. Exper. Station collection.

**12 *Micrathetis triplex* Walker**

Det. C. Heinrich. December 1936.

**13 *Atethmia subusta* Hübner**

Det. C. Heinrich. December 1936.

**14 *Atethmia oula* Dyar**

Det. C. Heinrich. December 1936.

**15 *Cobubatha quadrifera* Zeller**

Det. C. Heinrich. December 1936.

**16 *Paectes obrotunda* Guenee**

Det. C. Heinrich. December 1936.

**17 *Paectes* sp.**

Det. C. Heinrich. December 1936.

**18 *Pelamia repanda* F.**

Det. J. F. Gates Clarke. September 1936.

**19 *Phytometra ni* Hübner**

Det. L. O. Howard. Exp. Station collection.



- 20 **Phytometra** oo Cramer  
Det. L. O. Howard. Exp. Station collection.
- 21 **Melipotis contorta** Guenée  
Det. C. Heinrich. December 1936.
- 22 **Erebus odora** Linn.  
Det. C. Heinrich. November 1936.
- 23 **Concana disgrega** Möschler  
Det. C. Heinrich. December 1936.
- 24 **Concana** sp.  
new? Det. C. Heinrich. December 1936.
- 25 **Noropsis hieroglyphica** Cramer  
Det. C. Heinrich. November 1936.
- 26 **Alabama argillacea** Hübner  
Det. L. O. Howard. Exp. Station collection
- 27 **Gonodonta nitidimacula** Guenée  
Det. C. Heinrich. December 1936.
- 28 **Anticarsia gemmatalis** Hübner  
Det. C. Heinrich. December 1936.
- 29 **Epidromia pannosa** Guenée  
Det. L. O. Howard. Exp. Sta. collection.

## SPHINGIDAE

- 1 **Herse cingulata** Fabr.  
November 1936.
- 2 **Phlegethontius rusticus rusticus** Fabr.  
November 1936.
- 3 **Phlegethontius sextus jamaicensis** Butler  
November 1936.
- 4 **Erinnyis alope** Drury  
November 1936.
- 5 **Erinnyis ello** Linn.  
November 1936.
- 6 **Pholus vitis vitis** Linn.  
November 1936.
- 7 **Xylophanes pluto** Fabr.  
November 1936.
- 8 **Xylophanes tersa** Linn.  
November 1936.
- 9 **Celerio lineata lineata** Fabr.  
November 1936.

## GEOMETRIDAE

- 1 **Semiothisa paleolata** Guenée  
Det. H. W. Capps. January 1937.
- 2 **Racheospila** sp.  
Det. H. W. Capps. January 1937.

## PYRALIDAE

*Pyraustinae*

- 1 **Zinckenia fascialis** Cramer  
Det. L. O. Howard. Exp. Sta. coll.
- 2 **Syngamia florella** Cramer  
Det. C. Heinrich. November 1936.
- 3 **Pilocrocis tripunctata** F.  
Det. C. Heinrich. November 1936.
- 4 **Mesocondyla concordalis** Hübner  
Det. C. Heinrich. December 1936.
- 5 **Dichogramma fernaldi** Möschler  
Det. C. Heinrich. November 1936.
- 6 **Sylepta onophasalis** Walker  
Det. C. Heinrich. December 1936.
- 7 **Sylepta gordialis** Guenée  
Det. L. O. Howard. Exp. Sta. Coll.
- 8 **Sylepta patagialis** Zeller  
Det. L. O. Howard. Exp. Sta. coll.
- 9 **Lygropia flavofuscalis** Smellen  
Det. L. O. Howard. Exp. Sta. coll.
- 10 **Margaronia phlegia** Cramer  
Det. L. O. Howard. Exp. Sta. coll.
- 11 **Margaronia elegans** Möschler  
Det. L. O. Howard. Exp. Sta. coll.
- 12 **Margaronia immaculalis** Guenée  
Det. L. O. Howard. Exp. Sta. coll.
- 13 **Margaronia hyalinata** Linn.  
Det. L. O. Howard. Exp. Sta. coll.
- 14 **Margaronia costata** F.  
Det. C. Heinrich. November 1936.
- 15 **Ommatospila marcaeusalis** Walker  
Det. L. O. Howard. Exp. Sta. coll.
- 16 **Sameodes mopsalis** Walker  
Det. C. Heinrich. December 1936.

- 17 **Psara phaeopteralis** Guenée  
Det. L. O. Howard. Exp. Sta. coll.
- 18 **Psara bipunctalis** F.  
Det. L. O. Howard. Exp. Sta. coll.
- 19 **Noctuelia thalialis** Walker  
Det. L. O. Howard. Exp. Sta. coll.
- 20 **Leucinodes** sp.  
Det. C. Heinrich. December 1936.
- 21 **Loxostege bifidalis** Fabr.  
Det. L. O. Howard. Exp. Sta. coll.
- 22 **Hapalia vinotinctalis** Hampson  
Det. L. O. Howard. Exp. Sta. coll.
- 23 **Pyrausta phoenicealis** Hübner  
Det. L. O. Howard. Exp. Sta. coll.

*Chrysauginae*

- 1 **Pachymorphus subductellus** Möschler  
Det. C. Heinrich. November 1937.

*Crambinae*

- 1 **Diatraea saccharalis** F.  
Det. C. Heinrich. September 1936.
- 2 **Argyria diplomachalis** Dyar  
Det. C. Heinrich. November 1936.

*Phyctinae*

- 1 **Hyalospila** sp.  
Det. C. Heinrich. November 1937.
- 2 **Plodia interpunctella** Hübner  
Det. L. O. Howard. Exp. Sta. coll.
- 3 **Ephesiodes** sp.  
Det. C. Heinrich. October 1936.

HYBLAEIDAE

- 1 **Hyblaea puera** Cramer  
Det. C. Heinrich. September 1936.

YPONMEUTIDAE

- 1 **Plutella maculipennis** Curtis  
Det. L. O. Howard. Exp. Sta. coll.

## COSMOPTERYGIDAE

- 1 **Pyroderces rileyi** Walsingham  
Det. L. O. Howard. Exp. Sta. coll.

## BLASTOBASIDAE

- 1 **Blastobasidae** gen & sp.  
Det. C. Heinrich. November 1936.

## APOSTEGIDAE

- 1 **Apostega** sp. probably **trinidadensis** Busck  
Det. J. F. Gates Clarke. 1937.

## GELECHIIDAE

- 1 **Sitotroga cerealella** Olivier  
Det. L. O. Howard. Exp. Sta. coll.  
2 **Aristotelia** sp.  
Det. C. Heinrich. August 1936.  
3 **Pectinophora gossypiella** Saunders  
Det. L. O. Howard. Exp. Sta. coll.

## HYMENOPTERA

*Ichneumonoidae*

## BRACONIDAE

- 1 **Opius (Utetes) anastrephae** Viereck  
Det. F. W. Muesebeck. Reared from larvae of *Anastrepha mombin-praeoptans*. August 1939.  
2 **Apanteles leucostigma** Ashmead  
Det. F. W. Muesebeck. Reared from larvae of *Urbanus proteus*. September 1936.  
3 **Apanteles** n. sp.  
Det. F. W. Muesebeck. Reared from larvae of Lepidoptera in seed pod of mangle (*Rhizophora*). August 1936.  
4 **Chelonus** sp.  
Det. A. B. Gahan. January 1938.

## EVANIIDAE

- 1 **Evania appendigaster** Linn.  
Det. R. A. Cushman: Reared from egg-case of *Periplaneta americana*. October 1938.  
2 **Hyptia** sp.  
Det. R. A. Cushman. Swept from shrubbery. August 1937.

## ICHNEUMONIDAE

Determinations by R. A. Cushman.

- 1 **Labena** sp.  
Swept from grass. LaGrange. July 1936.
- 2 **Labena** n. sp.  
Collected by light on coco leaves. Crique. July 1936.
- 3 **Labena** n. sp.  
Swept from shrubbery. LaGrange. July 1936.
- 4 **Enicospilus concolor** Cresson  
At light. August 1936.
- 5 **Enicospilus** sp.  
August 1936.
- 6 **Ophion ancyloneura** Cameron  
At light. August 1936.
- 7 **Ophion** sp.  
At light. August 1936.
- 8 **Eiphosoma annulatum** Cresson  
Swept from grass. September 1936.
- 9 **Carinodes phavanensis** Cameron  
Swept from grass. August 1936.
- 10 **Pristomerus** sp.  
Swept by net. September 1937.

*Cynipoidea*

## CYNIPIDAE

- 1 **Hexacola** sp.  
Det. L. H. Weld. Swept by net. July 1940.
- 2 **Pseudeucoila** sp.  
Det. L. H. Weld. Swept by net. July 1940.

## FIGITIDAE

Determinations by L. H. Weld.

- 1 **Dicerataspis** sp.  
On rotting fruit. Fountain, July 1938.
- 2 **Eucoila (Hexamerocera) atriceps** Ashmead  
Reared from larvae of *Anastrepha mombinpraeoptans*, LaGrange.  
July 1938.

## TETRASTICHIDAE

- 1 **Tetrastichus hagenowi** Ratzeburg  
Det. A. B. Gahan. August 1937.
- 2 **Tetrastichus** sp.  
Det. A. B. Gahan. Reared from a spider egg-case. October 1938.

## ENTEDONTIDAE

- 1 **Horismenus** sp.  
Det. A. B. Gahan. Reared from its pupae attached to a leaf of *Rhizophora* (mangle). Krause Lagoon. December 1938.

## PTEROMALIDAE

- 1 **Neocatalaccus** sp.  
Det. H. K. Townes. Reared from galls of *Cocoloba wifera*. July 1938.

## ENCYRTIDAE

- 1 **Hunterellus hookeri** Howard  
Det. A. B. Gahan. Reared from female nymphs of *Rhipicephalus sanguineus*. October 1937.

## EUPELMIDAE

- 1 **Eupelmus** sp.  
Det. A. B. Gahan. Reared from seed pod of *Rhizophora*. June 1938.

## CHALCIDIDAE

Determinations by A. B. Gahan.

- 1 **Chalcis robusta** Cresson  
Swept from grass. July 1937.
- 2 **Brachymeria ovata** Say  
Swept from grass. September 1937.
- 3 **Brachymeria robustella** Wolcott  
Swept from grass. September 1937.
- 4 **Spilochalcis femorata** F.  
Swept from shrubbery. September 1937.
- 5 **Spilochalcis** sp.  
Swept from grass. December 1937. Reared from pupa attached to leaf of *Rhizophora*. Krause Lagoon. December 1938.

*Formicoidea*

Determinations by M. R. Smith. Specimens in the U.S.N.M.

*Ponerinae*1 *Platythyrea punctata* F. Smith

In decaying log. Foraging on tree trunk at night. Colony under scaling bark, 150 adults and some eggs, La Grange. March 1935.

2 *Ponera opaciceps* Mayr

A colony of 25 adults, eggs and pupae, under a large rock, Orange Grove. September 1938.

3 *Ponera ergatandria* Forel

Foraging. June 1938. Colony of 125 and 1 winged adult in decaying log, Prosperity. April 1938.

4 *Ponera* sp.

Adults taken foraging. December 1937.

5 *Anochetus mayri* Emery

Adults inside a dead palm trunk, Crique. 1938. Under a decaying log, Caledonia. November 1938. Foraging, Jealousy streamway. July. In moist pith of rotting log, Prosperity. April 1938.

6 *Odontomachus haematodes* Linn.

Colony of 50 adults and 20 winged forms, eggs and pupae, in a mass of rotting vegetable matter accumulated in a tree hole. When held between the fingers the adult would audibly snap its mandibles viciously. Mt. Victory. April 1935. Foraging on tree trunk at night. August 1935.

7 *Odontomachus haematodes insularis* Guérin

Colony of 4 winged adults, 50 workers and brown pupae beneath a board resting on the ground, Coakley Bay (semi-arid region). May 1938. A colony in a mound nest in the fork of two large branches, Collins. July 1938. Foraging at night. July 1938. A colony of 50 and 15 winged adults, pupae and eggs, under loose bark, Mt. Victory. November 1943.

8 *Odontomachus* sp.

Winged adults taken at light. Annas Hope. August 1937.

*Myrmicinae*1 *Monomorium carbonarium ebeninum* Forel

Adults foraging on decaying lilly bulbs, Oxford. 1935. Winged adults at light. December 1937. Colony of 15 winged adults, 150 workers, pupae and eggs, under the scaling bark of a tree stump, Jealousy streamway. January 1937. Colony of 500 adults, eggs and pupae in a decaying stump. September 1938. A colony of approximately 2000 adults, eggs and pupae, in a dry length of *Cecropia*. October 1938.

**2 *Monomorium destructor* Jerdon**

Attracted to food, Coakley Bay (semi-arid). At light, winged adults. December 1937.

**3 *Monomorium floricola* Jerdon**

Among rotting debris, Caledonia. March 1938. Adults were observed carrying off the eggs of *Aedes aegypti* from a tub. July 1935. A colony of 300 adults, eggs, and pupae, in the dry branch of avocado tree, Kingshill. July 1935.

**4 *Cardiocondyla emeryi* Forel**

Adults foraging, Prosperity. June 1935. Adults beneath dry cattle dung, Butler's Bay. March 1937. On flowers of cactus, *Opuntia* sp., Sight. May 1938.

**5 *Cardiocondyla venustula* Wheeler**

July 1936.

**6 *Solenopsis corticalis* Forel**

A small colony beneath a large rock, Prosperity. 1938.

**7 *Solenopsis geminata* Fabr.**

Tunnels in sandy soil, Krause Lagoon. April 1935. Winged adults at light. August–December, 1937. Under a log on the sea shore. A ground nest of approximately 2000 workers and 100 winged adults, besides eggs and pupae, Oxford. March 1938.

**8 *Solenopsis globularia borinquenensis* Wheeler**

Foraging on gravelly soil Oxford. April 1935. Winged adults swarming at dusk, Lower Love. January 1941.

**9 *Solenopsis* sp.**

Adults foraging, Christiansted cemetery. June 1935. Winged adults at light. June 1935. Winged male and female were taken in copula in flight at dusk, Sight. November 1935. Winged adults at light, Mary's Fancy. February 1936. Colony in decaying stump, eggs. September 1938. Winged adults swarming at dusk, La Grange. June 1941.

**10 *Pheidole fallax jelskii* var. *antillensis* Forel**

A colony of approximately 700 workers and 26 winged forms in a ground nest. They have a predominantly fecal odor. Prosperity. April 1938.

**11 *Pheidole flavens sculptior* Forel**

Colony of 150 adults, pupae and eggs, in pith of rotting log, Prosperity. April 1938. Foraging on moss, Caledonia. December 1940.

**12 *Pheidole flavens thomensis* Emery**

Colony beneath a large rock, Crique. May 1937. Winged adults at light. December 1937.



- 13 **Pheidole moerens** Wheeler  
Adults under dry cattle dung, Butler's Bay. March 1937. Colony in gravelly soil beneath a large rock, Concordia. May 1938.
- 14 **Pheidole megacephala** Fabr.  
Colony in a hole in wall, several queens were noted. These ants emit an audible squeak while actively foraging. At dusk swarms were flying against a light breeze and were being captured and eaten by Kingbirds, *Tyrannus dominicensis*, and Anis, *Crotophaga ani*. Beeston Hill. February 1936. Winged adults at light. December 1937.
- 15 **Pheidole** sp. **flavens** group  
Adults foraging, Prosperity. June 1935. Male and female in copula taken in flight, Constitution Hill. December 1935. Winged adults taken in flight. February 1936.
- 16 **Crematogaster steinheili** Forel  
Colony of 200 adults, pupae and eggs, in dry tree branch, and when disturbed they ran around with the gaster held erect, Oxford. April 1935. Winged adults at light, Mary's Fancy. February 1936. Colony in rotting branch, Annaly. February 1935.
- 17 **Tetramorium guineense** Fabr.  
Adults were foraging on water-hyacinth plants in Annas Hope stream. July 1935.
- 18 **Tetramorium lucayanum** Wheeler  
Adults taken foraging, Caledonia. December 1937 and December 1940.
- 19 **Tetramorium simillimum** Nylander  
Foraging. December 1937. Adults in rotting stump, Oxford. April 1937.
- 20 **Wasmannia auropunctata** Roger  
Colony in cavity of dry branch, Mt. Eagle. June 1935. Colony of 200 adults, pupae and eggs, under scaling bark, La Grange. March 1936. Adults, Caledonia. December 1940.
- 21 **Leptogenys puniticeps vincentensis** Forel  
Adults were taken foraging at night, Morning Star pump. July 1938.
- 22 **Strumigenys eggersi** Emery  
Winged adults were taken in a house in Christiansted. June 1935.
- 23 **Strumigenys rogeri** Emery  
Adults taken foraging on moss, Caledonia. December 1940.
- 24 **Myocepurus smithi borinquensis** Wheeler
- 25 **Epitritus emmae** Emery
- 26 **Cyphomyrmex rimosus minutus** Mayr  
Colony of 200 adults, pupae and eggs, in moist soil near a water

hole, Lower Love. February 1936. Colony beneath a large rock, 150 and 15 winged adults, pupae and eggs. March 1937. Colony of 50 adults, pupae and eggs, in moist soil beneath a large stone. When disturbed the adults play possum and remain quiet for several minutes. April 1938.

*Dolichoderinae*

1 **Tapinoma melanocephalum** Fabr.

Pests in insectory stealing everything. However, it is not widely distributed. July 1935.

2 **Iridomyrmex melleus** Wheeler

A small colony taken from a dry branch, Mt. Eagle. June 1935.

3 **Dorymyrmex pyramicus niger** Pergande

A colony of 600 adults, pupae and eggs. Very active when disturbed, tunneling in soil. Several mounds that were five feet apart were connected by overland trails and the ants were seen moving from one to the other. Slob Bay (semi arid area). May 1935. Colony in the cavity of a dry branch. March 1938.

*Formicinae*

1 **Brachymyrmex heeri** Forel

A colony of 3 winged adults and 35 workers in a root-node cavity, Christiansted, September 1935. Adults foraging, Oxford. April 1935. Small colony beneath a rock, Grange streamway. A small colony beneath scaling bark, Collins. July 1938.

2 **Brachymyrmex** sp.

Swarming winged adults in flight at dawn, Blessing. February 1936. Adults foraging, LaGrange. June 1943.

3 **Prenolepis (Nylanderia) fulva** Mayr

Foraging adults were taken about the edge of a small pool, Blessing, July 1935. Feeding on fruit of *Cactus intortus*, Sight. July 1936.

4 **Prenolepis (Nylanderia) vividula** Nylander

Foraging adults. July 1935.

5 **Prenolepis (Nylanderia) longicornis** Latreille

Colony of 50 winged adults and 500 workers beneath a log on the sandy beach, Watch Ho. September 1938.

6 **Prenolepis (Nylanderia)** sp.

Adults taken foraging, La Grange. December 1937.

7 **Camponotus sexguttatus** Fabr

Colony of 300 adults, eggs and pupae, in the cavity of a dry branch, Constitution Hill. April 1935. Colony of pupae and eggs, 75 workers and 18 winged adults, beneath scaling bark. September 1938.

8 **Camponotus** sp.

Colony of 300 in a hollow stump, Green Cay Islat. January 1936.  
Colony in decaying log, Prosperity. May 1937. A colony of pupae  
and eggs, 200 workers and 18 winged adults, beneath a rotting log  
resting in sand, Coakley Bay (semi-arid). May 1938.

9 **Myrmelachista ramulorum** Wheeler

Adults taken foraging on mangroves, Krause Lagoon. May 1935.

10 **Rhizomyrma** sp.

Winged adult captured in flight at dusk, Constitution Hill, October  
1937.

*Sphecoidea*

## BEMBECIDAE

Determinations by G. A. Sandhouse.

1 **Bembex muscicapa** Handlirsch

Taken at tunnels in sandy soil. August 1936.

2 **Stictia signata** Linn.

Predaceous on *Hermetia illucens*, *Trichopoda* sp, *Lucilia* sp. Tunnels  
in sandy soil. September 1936.

## SPHECIDAE

1 **Notogonidea trifasciata** Smith

Det. K. V. Krombein. September 1936.

2 **Notogonidea vinulenta** Cresson

Det. K. V. Krombein. September 1939.

3 **Notogonidea** sp.

Det. K. V. Krombein. September 1939.

4 **Tachytes argentipes** Smith

Det. K. V. Krombein. July 1937.

5 **Prionyx thomae** Fabr.

Det. K. V. Krombein. August 1936.

6 **Tachysphex** sp.

Det. G. A. Sandhouse. August 1937.

7 **Ammobia singularis** F. Smith

Det. G. A. Sandhouse. Males and females taken from tunnels in  
soil, Adventure. August 1936.

*Vespoidea*

## SCOLIIDAE

1 **Myzine ephippium** Fabr.

Det. G. A. Sandhouse. August 1937.

2 **Campsomeris dorsata** Fabr.

Det. G. A. Sandhouse. September 1937.

## PSAMMOCHARIDAE

1 **Pepsis rubra** Drury

Det. J. Bequaert. Tunnels in limestone cliffs. August 1935.

2 **Batazonus mundiformis** Rohwer

Det. K. V. Krombein. July 1936.

3 **Planiceps** sp.

"Probably new." Det. K. V. Krombein. September 1937.

## VESPIDAE

1 **Polistes crinitus insulicola** Beq. & Sal.

Det. J. Bequaert. Predaceous on small lepidopterous larvae. Nesting in October 1935.

2 **Mischocyttarus phthisicus** Fabr.

Det. J. Bequaert. Nests October 1935.

## EUMENIDAE

1 **Pachodyneurus (Monobiella) cinerascens** Fabr.

Det. J. Bequaert. Tunnels in limestone cliffs, July 1935. Predaceous on lepidopterous larvae.

2 **Odyneurus** sp.

Det. K. V. Krombein. July 1935.

*Apoidae*

## HALICTIDAE

1 **Halictus busckii** Cockerell

Det. K. V. Krombein. June 1936.

2 **Halictus** sp.

Det. K. V. Krombein. July 1936

3 **Halictus** sp.

Det. K. V. Krombein. July 1936.

## ANTHOPHORIDAE

1 **Centris poecila** Lepelletier

Det. G. A. Sandhouse. October 1937.

2 **Centris versicolor** Fabr.

Det. K. V. Krombein. October 1937.

3 **Exomalopsis globosa** Fabr.

Det. K. V. Krombein. September 1936.

**4 Anthophora krugii** Cresson

Det. K. V. Krombein. August 1936.

## EUCERIDAE

**1 Melissodes trifasciata** Cresson

Det. K. V. Krombein. June 1935.

## MEGACHILIDAE

Determinations by G. A. Sandhouse.

**1 Coelioxys abdominalis** Guérin

July 1936.

**2 Megachile binotata** Guérin

On flowers. August 1936.

**3 Megachile flavitarsata** Smith

On flowers. August 1936.

## XYLOCOPIDAE

**1 Xylocopa brasilianorum** Linn.Det. K. V. Krombein. Tunnels in dry stump of *Hippomane mancinella*. September 1935.

## BRACHYURAN CRABS OF ST. CROIX, V. I.

By HARRY A. BEATTY

In a paper on the brachyuran crabs, "Scientific Survey of Porto Rico and the Virgin Islands," Vol. XV, part 1, 1933, Rathbun recorded 47 species from St. Croix. The Beatty collection was brought together during 1933 to 1938. It catalogs 53 species, of which 37 are new records, and raises the number of crabs reported from the island to 68 species, distributed among 50 genera and 13 families. The collection was donated to the National Museum, and the author is indebted to Waldo L. Schmitt for making the determinations.

It is quite well known that the transference of living organisms from a salt-water environment to another of purely fresh water will produce fatal functional results. Yet, here we are confronted with an adaptation by organisms to suit their caprices, we concede this since there are no other compelling forces in evidence, the Fairplain complex presents a field of such unreasonable surprises.

Let us voyage up the streamway, beginning at the seacoast, picking up such things as *Callinectes sapidus acutidens*, *Panopeus herbstii forma crassa*, *Pachygrapsus transversus* in the mossy shallows. The waters around us is teeming with fish, including such forms as the mullet, cramo, snook, heddo, yellowtail and tarpon. As we travel along upstream, preferably in a batteau, we notice that the water is rapidly changing, and throughout the transition from salt to fresh we find *Panopeus* clinging to clumps of moss enveloping the roots of the mangrove (*Avicennia* sp.); *Callinectes* can be coaxed up from the deep to the water's edge by a mash prepared from crushed land crabs (*Cardisoma guanhumi*). We are now half of the way to the bridge, which is six hundred yards from the sea, the stream at this point is six feet deep, the dark brown liquid is about twenty-five per cent salt water. Beyond this point the stream bed is ascending, the water freshens rapidly, and is from one to three feet in depth. It is impossible to send the batteau against the current, so from here we must wade, bypassing many sizable pools between banks fringed with black mangroves (*Rhizophora*); until the bridge forms a barrier, where the stream cascades over a high pavement. Here, in contrast, the water is fresh and clear. We are more than ever eager to explore; so overboard another generous offering of mash and up comes *Callinectes sapidus acutidens*, *C. bocourti*, to be tricked into a net. In the evening we can surprise *Callinectes* at the water's edge, audibly snapping its chela at passing shrimps, and making occasional captures. Now, wading in along the pools and turning up the larger rocks there, again you see *Panopeus herbstii forma crassa*, *Pachy-*

*grapsus transversus* and *P. gracilis* hurriedly shifting themselves to hide in the gravelly bed. From the pools, frightened mullet, cramo, snook and tarpons dart downstream. In the becalmed back-wash of the flow small heddo and yellowtails are poised motionless in readiness to pounce upon unwary victims. Here, also, roams the delicate little pipefish *Doryrhamphus lineatus* (E. Reid).

And there is another picture of nature at her best.

#### MAJIDAE

- 1 ***Stenorynchus sagittarius*** Fabr.  
On moss covered coastal rocks. Christiansted harbor.
- 2 ***Anomalothir furcillatus*** Stimpson  
115 fathoms.
- 3 ***Euprognatha gracilipes*** A. M. Edwards  
Frederiksted harbor.
- 4 ***Acanthonyx petiverii*** H. M. Edwards  
Frederiksted harbor, in moss.
- 5 ***Espialtus bituberculatus*** H. M. Edwards  
Shallows, in moss. Salt River.
- 6 ***Menaethiops portoricensis*** Rathbun  
Frederiksted harbor, shallows, moss.
- 7 ***Trachymaia cornuta*** A. M. Edwards  
82 fathoms.
- 8 ***Chorinus heros*** Herbst  
In moss, Salt River.
- 9 ***Pitho lherminieri*** Schramm
- 10 ***Mithrax (Mithrax) acuticornis*** Stimpson  
12-45 fathoms.
- 11 ***Mithrax (Mithrax) holderi*** Stimpson
- 12 ***Mithrax (Mithrax) pilosus*** Rathbun  
4 fathoms, in fish traps.
- 13 ***Mithrax (Mithrax) hispidus*** Herbst  
4 fathoms, in fish traps.
- 14 ***Mithrax (Mithrax) caribbaeus*** Rathbun  
Frederiksted harbor.
- 15 ***Mithrax (Mithrax) pleuracanthus*** Stimpson  
15 fathoms, off Coakley Bay.
- 16 ***Mithrax (Mithraculus) sculptus*** Lamarek  
Coastal rocks, Judith Fancy Bay.
- 17 ***Mithrax (Mithraculus) coryphe*** Herbst  
Shallows, moss, Frederiksted harbor.

- 18 **Mithrax (Mithraculus) forceps** A. M. Edwards  
15 fathoms, Coakley Bay.
- 19 **Teleophrys ornatus** Rathbun  
4 fathoms.
- 20 **Macrocoeloma diplacanthum** Stimpson
- 21 **Macrocoeloma eutheca** Stimpson  
37 fathoms.
- 22 **Microphys bicornutus** Latreille  
Shallows, moss, Judith Fancy Bay.

## PARTHENOPIDAE

- 1 **Solenolambrus tenellus** Stimpson  
35 fathoms.
- 2 **Heterocrypta** sp.  
Moss, Frederiksted harbor.

## PORTUNIDAE

- 1 **Portunus (Achelous) sebae** H. M. Edwards  
2 fathoms, Christiansted harbor.
- 2 **Callinectes sapidus acutidens** Rathbun  
At bridge, Fairplain stream.
- 3 **Callinectes ornatus** Ordway  
Shallows, Salt River Bay.
- 4 **Callinectes marginatus** A. M. Edwards  
Shallows, Salt River Bay.
- 5 **Callinectes bocourti** A. M. Edwards  
At bridge, Fairplain stream.
- 6 **Callinectes exasperatus** Gerstaecker  
4 fathoms, Christiansted harbor.

## POTAMONIDAE

- 1 **Epilobocera sinuatifrons** A. M. Edwards  
Caledonia stream.

If *sinuatifrons* communicate among themselves by subterranean signals then there is a measure of eager anticipation awaiting the patient student who would decipher the code. The crab may shelter beneath rocks and within cracks by day, and often prepares its own hole in loose soil, but, always, it is found in shaded mountain streams. Comes nightfall, and the patient listener is suddenly thrilled to a muffled drumming: tat tat tat, tat. Tat, tat, tat, tat, and so continuing for many long minutes. Presently a reply comes from across the way and thereafter the signals come drifting in from numerous points. The manner by



which the sound is produced remains unknown but apparently it is made before coming into the open, although roaming crabs might be expected to carry on. Any attempt on the part of the listener to draw closer conveys a warning as, also, a beam of light and instantly the signals fade into the tranquility of the jungle's shadows.

## XANTHIDAE

- 1 **Carpilius corallinus** Herbst  
5 fathoms, off the north coast.
- 2 **Actaea setigera** H. M. Edwards  
Shallows, moss, Frederiksted Harbor.
- 3 **Actaea rufopunctata nodosa** Stimpson  
70 fathoms.
- 4 **Actaea acantha** H. M. Edwards  
Shallows, moss, Frederiksted harbor.
- 5 **Leptodius floridanus** Gibbs  
Shallows, under rocks, Salt River Bay.
- 6 **Xantho** sp.  
Near nudipes. Shallows, Green Cay Islet.
- 7 **Panopeus herbstii** H. M. Edwards  
Shallows, Salt River Bay.
- 8 **Panopeus herbstii forma crassa** A. M. Edwards  
In moss and beneath rocks, Fairplain stream below bridge.
- 9 **Panopeus herbstii forma simpsoni** Rathbun  
Moss, Frederiksted harbor.
- 10 **Panopeus occidentalis** Saussure
- 11 **Panopeus bermudensis** Benedict & Rathbun  
3 fath. Christiansted harbor.
- 12 **Eurypanopeus abbreviatus** Stimpson
- 13 **Micropanope sculptipes** Stimpson  
15 fathoms.
- 14 **Micropanope lobifrons** A. M. Edwards  
80 fathoms.
- 15 **Micropanope pusilla** A. M. Edwards  
17 fathoms.
- 16 **Micropanope urinator** A. M. Edwards  
245 fathoms.
- 17 **Micropanope barbadensis** Rathbun  
Shallows, moss, Frederiksted harbor.
- 18 **Chlorodiella longimana** H. M. Edwards
- 19 **Pilumnus marshi** Rathbun
- 20 **Pilumnus reticulatus forma fragosa** A. M. Edwards

- 21 **Ozium reticulatus** Desbonne & Schramm  
Shallows, Judith Fancy Bay.
- 22 **Eriphia gonagra** Fabr

## CYMOPOLIIDAE

- 1 **Cymopolia affinis** A. M. Edwards  
115 fathoms.
- 2 **Cymopolia sica** A. M. Edwards  
20 fathoms.
- 3 **Cymopolia angusta** Rathbun  
117 fathoms.
- 4 **Cymopolia depressa** Rathbun  
117 fathoms.

## GRAPSIDAE

- 1 **Grapsus grapsus** Linn  
Shallows, under rocks, Green Cay.
- 2 **Geograpsus lividus** H. M. Edwards  
Shallows, under rocks, Green Cay.
- 3 **Goniopsis cruentata** Latreille  
Green Cay.
- 4 **Pachygrapsus transversus** Gibbs  
Shallows, Salt River Bay. Under rocks, Fairplain stream below bridge.
- 5 **Pachygrapsus gracilis** Saussure  
Under rocks, Fairplain stream, bridge.
- 6 **Pachygrapsus corrugatus** Von Martens  
Shallows, Judith Fancy Bay.
- 7 **Sesarma (Holometopus) ricordi** H. M. Edwards  
Along the coast.
- 8 **Sesarma (Holometopus) angustipes** Dana  
Along the coast.
- 9 **Cyclograpsus integer** H. M. Edwards  
Shallows, Judith Fancy Bay.
- 10 **Plagusia depressa** Fabr  
About mangroves near swamps.
- 11 **Percnon gibbesi** H. M. Edwards

## GECARCINIDAE

- 1 **Cardisoma guanhumi** Latreille  
Common land crab.

# THE FRESH WATER FISHES OF ST. CROIX, V. I.

By HARRY A. BEATTY

Of the numerous small streams which seam the island from the highlands to the seacoast only a few of them, under normal conditions, maintain running water throughout the year. Nevertheless, the finny tribes inhabiting them survive the drought in some miraculous way and with the rains they again reappear in the form of larval fishes to populate the streams. This is particularly true of *Sicydium plumieri* which can be seen hovering near her eggs which are attached to the sheltered side of a rock in swift running water. This beautiful little fish, of steel-blue with white fins, will desert her spawn with the greatest reluctance when disturbed but will return immediately to stand guard. The determinations in the paper were made by Earl D. Reid and a few by the writer. The specimens are deposited in the U. S. National Museum.

## GINGLYMOSTOMIDAE

### 1 *Ginglymostoma cirratum* Gmelin

Nurse-shark frequently seen in the slightly brackish waters of Fairplain stream a quarter mile from its entrance into the sea.

## MYLIOBATIDAE

### 2 *Aetobatus narinari* Euphrasen

Small specimens are often seen in the slightly brackish waters of Fairplain stream.

## ANGUILLIDAE

### 3 *Anguilla bostonensis*

Det. E. D. Reid. During the winter months the larval eel, which is about two inches in length, can be seen migrating upstream in Fairplain, Concordia and Caledonia streams.

## ELOPIDAE

### 4 *Tarpon atlanticus* Cuvier & Valen

Large specimens enter the slightly brackish waters of Fairplain stream a quarter mile from the sea.

## ALBULIDAE

### 5 *Albula vulpes* Everman & Marsh

In South Gate Pond; water brackish.

## POECILIIDAE

6 **Lebistes reticulatus**

Common in all streams and reservoirs. Omnivorous in feeding habits.

## SYNGNATHIDAE

7 **Doryrhamphus lineatus**

Det. E. D. Reid. Taken in Fairplain stream, below bridge. Feed on minute aquatic creatures. Rare.

## MUGILIDAE

8 **Mugil brasiliensis** Everman & Marsh

Enters fresh-water streams.

9 **Mugil curema** Everman & Marsh

Enters fresh-water streams.

10 **Agonostomus monticola** Everman & Marsh

Det. E. D. Reid. A species that is never abundant but has an extremely interesting life cycle which requires investigation. It would be of interest to explain how streams that have become dust beds and may remain in that condition for as many as three successive years can become populated with minute larval fish about three months after inundation by heavy rainfall. This species subsists entirely on animal matter.

## CENTROPOMIDAE

11 **Centropomus undecimalis** Everman & Marsh

Common in the waters of Fairplain stream. Feeds on animal matter.

## GOBIIDAE

Det. E. D. Reid

12 **Philypnus dormitor** Everman & Marsh

Taken in Altona, Concordia and Fairplain streams.

13 **Dormitator maculatus** Bloch

Shoys Marsh, Fairplain stream. Feeds on animal matter.

14 **Sicydium plumieri** Bloch

Caledonia stream. Feeds on moss.

15 **Evorthodus lyricus**

Caledonia and Fairplain streams. Feeds on moss.

16 **Awaous taiaxica** Everman & Marsh

Fairplain and Concordia streams.

2 **Ucides cordatus** Linn

Rare, Morningstar swamp.

**3 Gecarcinus lateralis** Fremenville

Along the coast.

**4 Gecarcinus ruricola** Linn.

Green Cay Islet.

## OCYPODIDAE

**1 Ocypode albicans** Bosc

Common along the beach.

**2 Uca mordax** Smith

At Tagus Pond, Krause Lagoon, Longpoint pond.

**3 Uca pugnax rapax** Smith

Is found near fresh and brackish water generally, Fairplain stream, Rust-op-twist pond, and at Salt pond close to a spring.

## LEUCOSIIDAE

**1 Lithadia granulosa** A. M. Edwards

115 fathoms.

## CALAPPIDAE

**1 Calappa flammea** Herbst

Fish traps at 3 fathoms, Christiansted harbor

**2 Calappa angusta** A. M. Edwards

Fish trap, 3 fathoms, Christiansted harbor.

**3 Calappa gallus** Herbst

Fish traps, 3 fathoms, Christiansted harbor.

**4 Osachila antillensis** Rathbun

67 fathoms.

## DORIPPIDAE

**1 Cymonomus quadratus** A. M. Edwards

100 fathoms.

## DROMIIDAE

**1 Dromia erythropus** G. Edwards

30 fathoms off Buck Island.

**2 Dromidia antillensis** Stimpson

Frederiksted harbor.

## HOMOLIDAE

**1 Homologenus rostratus** A. M. Edwards

580 fathoms.

## THE MAMMALS OF ST. CROIX, V. I.

By HARRY A. BEATTY

The mammalian fauna was very poorly represented on St. Croix. Of 8 recorded species, 3 of them, *Capromys*, *Isolobodon* and *Dasyprocta*, became exterminated during historic times, and probably were influenced directly by the destruction of the original forests. The dispersal over the island of the three extinct species and their importance in the Carib economy is borne out by the abundance of remains found in kitchen middens. Later, followed the introduction of 4 species of exotic animals. The arrival of *Rattus* is unrecorded. The mongoose was thought to be the answer to the sugar cane planter's prayer for an efficient enemy of the rats which had overrun the plantations, becoming an important pest and doing considerable damage to the crops annually. Amid much ceremony and well wishing 3 pairs of mongoose were liberated on the island in 1867. There is evidence that the rats have been considerably reduced but they are yet here and still a serious pest, along with the mongoose. The deer was brought in from the continent by the captain of a trading schooner about the year 1790.

I am indebted to Alexander Wetmore for determinations of the fossil material and to Geret S. Miller, Jr., for the identifications of bats. Remington Kellogg very kindly arranged the orderly presentation of the nomenclature and identified the mongoose and the deer. The *Muridae* was determined by the writer.

### Class **MAMMALIA**

#### Subclass **Eutheria**

#### Order **CHIROPTERA** (Bats)

#### Family **NOCTILIONIDAE**

#### 1 **Noctilio leporinus mastivus** Dahl

I think this is one of the most highly odorous of the bats and a keen olfactory can detect the pungent musk as the animal passes by even though it may be fifty feet away. It would be unusual to find *Noctilio* in the woods but a trip to the nearest body of water, whether it is a pool, a large dam or in a quiet bay, will reveal the aptness with which it can pick up insects and small fish on the surface. For the purpose it has developed a skin pouch controlled by the hind limbs and whatsoever is scooped up is quickly transferred to the mouth and as the creature continues in graceful flight a distinct crunching sound can be heard as the food is chewed in powerful jaws. This bat is rare.

## Family PHYLLOSTOMIDAE

1 **Brachyphylla cavernarum** Gray

Small colonies seek shelter in old buildings in disuse or cluster together in dense tree tops. Common.

2 **Artibeus jamaicensis jamaicensis** Leach

This bat is often found in sizable colonies sheltering in old buildings side by side with *Brachyphylla* but the two species do not intermingle. Little bunches of five or ten can be seen hanging from the fronds of the coconut palm or in trees with heavy foliage. The female is often seen flying at dusk with her young clutching to the fur on her abdomen. A common species.

## Family MOLOSSIDAE

1 **Molossus major** Kerr

This the most common bat on the island can be seen at dusk high in the air, pursuing flying insects. During daylight it secretes itself in crevices and more often in the dark ceilings in houses. Both sexes have a musk gland beneath the chin and its strong pungent odor pervades its haunts.

## Order CARNIVORA

## Family VIVERRIDAE (Mongoose)

1 **Herpestes javanicus auropunctatus** Hodgson

The impact of this vicious little predator upon the avian fauna is reflected in the rapid numerical decrease of ground-nesting birds, notably the Bobwhite (*Colinus virginianus*), after its introduction into the island from India, via Jamaica, in 1867. It has become well dispersed, its habits are very simple and my recent studies have shown that it can be eradicated from the island. That it is an exceedingly healthy little beast is shown in an intensive search for diseased material which, so far, has been unsuccessful but it is pestered by fleas (*Ctenocephalides felis*) and an insatiable appetite. It begins its day hunting for lizards on the dew-laden grasses at dawn and may climax it with a bird or two at setting of the sun. As an omnivorous feeder it is notorious and is fond of live reptiles, snails, insects and small animals as well as decomposing tissue and ripe fruits. During the mating season a den is prepared in a hollow log or other natural cavity and there a pair of kittens is born in July-August. The mongoose rarely climbs into trees and will haunt the borders of a pond in its search for crabs and dead fish. Erik Lawaetz tells a story

of how his attention was attracted to a tall tree by the squeaking of a rat. He saw a mongoose rummaging in the bulky leaf nest of a rat when suddenly the rat ran out and quickly reentered. This was followed by agonizing squeaks after which the rat lurched from the nest and fell to the ground twenty-five feet below. It had been injured by the mongoose, but dragged itself into the dense underbrush and was lost. The mongoose presently emerged from the rat's nest and, as Erik says, "was licking his chops." From the behavior of both the rat and the mongoose it is probable that young rats in the nest were the victims. But the mongoose has its trials too for one day I saw a hawk (*Buteo jamaicensis jamaicensis*) pick up a half-grown young from atop a rock pile. Similar occurrences have been related by the hill folks.

## Order RODENTIA

### Family MURIDAE

#### 1 *Mus musculus musculus* Linnaeus

Common in the fields and woods, and about dwellings. No parasites have been recorded from this mouse.

#### 2 *Rattus rattus alexandrinus* Geoffroy

Tree rat. The nest of the tree rat is a familiar sight in the woods. It is constructed of green leaves that are loosely held together by the small branches clustered at the end of a limb; saplings and vines offer the best sites. But this rat is at home equally in the lagoons where the large bulky nest is placed in the small mangrove clumps often growing in isolated spots and the rat must swim to and fro on foraging expeditions. This nest is usually three feet in diameter and while the outside is quite dry, the core is always being replenished, almost nightly, with fresh green leaves. A test has shown that considerable humidity and a marked thermal increase are released by bacterial action on the green vegetable matter, creating an environment that must be essential to the physical comfort of the animal. When a nest is disturbed the rats flee instantly, plunging into the water and swimming below for twenty feet before surfacing.

#### 3 *Rattus norvegicus* Berkenhout

House rat. The common rat about buildings. It is notorious for the destruction of chickens and small mammals, as well as doing wide-spread damage to stored produce. The nest is constructed of any thing available and the preferred location is in a tree hole, in boxes in the cellar or in burrows in the ground.



## Family ECHIMYIDAE (Spiny Rats)

1 *Capromys* sp.

Skeletal material has been recovered from the kitchen middens in Carib settlements located in the western two thirds of the island which was heavily forested at the time.

2 *Isolobodon* sp.

Kitchen middens also yielded the only evidence known of the occurrence of the animal in the island.

## Family DASYPROCTIDAE (Agoutis)

1 *Dasyprocta aguti* Linnaeus

There are no recent records of the agouti in St. Croix but it is yet found in the forests on some neighboring islands. Kitchen midden material indicates that it was abundant during the Carib era, and the wide extent to which it was used as food. The passing of the agouti from the island is, probably, simultaneous with the destruction of the heavy forests during historic times and it became an easy prey of hunting dogs.

## Order ARTIODACTYLUS

1 *Odocoileus virginianus* subsp.

This form of the North American white-tailed deer has become happily adjusted to its environment on St. Croix, following its introduction in 1790. There is abundant evidence of its physical stamina and the examination of carcasses for internal parasites have yielded only the adult worms of *Cysticercus tenuicollis* which were attached to the mesenteries. This worm also has been reported from sheep in the island.

The deer is subjected to infestations with the cattle tick, *Boophilus annulatus microplus*. During a campaign devised for the extermination of this tick from the island the deer, it was agreed, was host to the tick and therefore a potential menace to the cleanup program and so was destined for a vigorous campaign of slaughter. In the course of the first twelve month period approximately one thousand two hundred deer were killed and many of them were carefully examined. All domestic animals, meanwhile, were subjected to dipping in arsenical solutions. At the close of the twelve month period I checked my field notes and observed that deer killed six months after dipping had begun were entirely tick-free and this record was maintained unmarred up until such time as the project was discontinued. Later, by a careful analysis of all the accumulated data, I was able to arrive at conclusions as following. It is wholly unnecessary to eradicate the deer on St. Croix in any attempt to make the island tick-free. The

only expedient suggested is an effort aimed at reducing the deer first to a numerical low level by increased hunting pressure and thereafter to maintain a continual movement of the deer in every section of the island. This could best be accomplished by a crew of beaters with dogs. By this method, the deer is not allowed to bed down in a favorite haunt which would be naturally infested with seed ticks. By frustrating the instinct of the seed tick to attach itself to a warm blooded host it will perish from the lack of nourishment within thirty days. Other stray ticks picked up by domestic animals were killed by dipping.

The rutting season for deer begins in June and is persistent until about September. The length of the gestation period is undetermined and it may be 120 days. The fawning season is from November to February when twins are born to the matured does and a single fawn is dropped by the young doe. The sex ratio of fawns is predominantly female and it is uncertain whether a set of twins will be of mixed or similar sexes. There is some evidence to bear out the belief that similar sexes, rather than the exception, is nearer the rule. The buck sheds his antlers during September-November and it is nothing unusual to see several of them ranging together at that time. The new antlers reach maturity about six months later. The yearling buck sprouts his spike horns at 7-8 months of age at a time when the fur is assuming the brown spotless coat of the adult. The exceptions at variance with this data are the records of fawns born in every month of the year. The post-seasonal fawning rate appears to be quite low, and is in itself a biological phenomena already observed among members of this family in the tropics. However, it is suspected that yearling does are principal offenders in these instances.



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